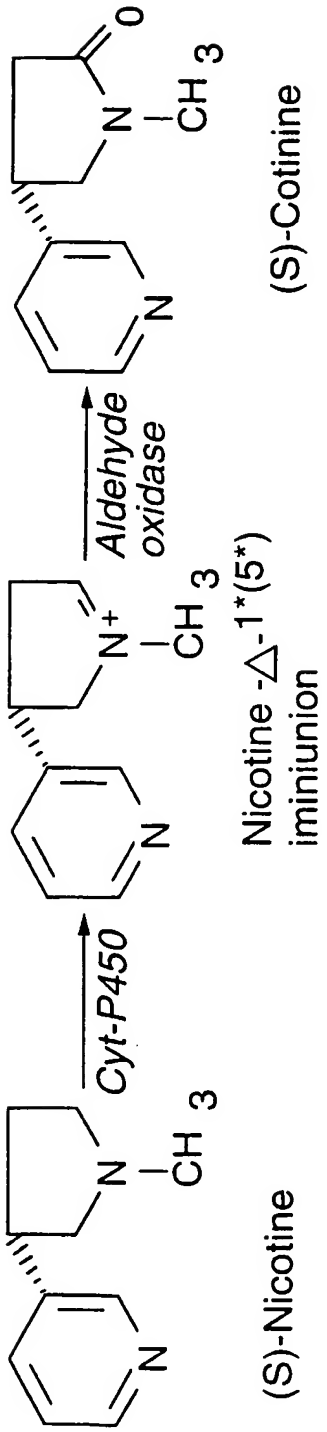

(S)-Nicotine
Cyt-P450
Nicotine -Δ-1*(5*) iminiunion
Aldehyde oxidase
(S)-Cotinine

FIG.1

FIG.2A

```
LOCUS       HSU22027     7215 bp      DNA          PRI       22-OCT-1995
DEFINITION  Human cytochrome P450  (CYP2A6V2)  gene, complete cds.
ACCESSION   U22027
NID         g1008461
KEYWORDS    .
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 7215)
  AUTHORS   Fernandez-Salguero,P., Hoffman,S.M., Cholerton,S.,Mohrenweiser,H.,
            Raunio,H., Rautio,A., Pelkonen,O., Huang, J.D., Evans, W.E.,
            Idle, J.R. et,al.
  TITLE     A genetic polymorphism in coumarin 7-hydrozylation: sequence of the
            human CYP2A genes and identification of variant CYP2A6 alleles
  JOURNAL   Am. J. Hum. Genet. 57 (3), 651-660 (1995)
  MEDLINE   95397851
REFERENCE   2 (bases 1 to 7215)
  AUTHORS   Fernandez-Salguero,P.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-MAR-1995)  Pedro Fernandez-Salguero,  National
            Institutes of Health, 9000 Rockville Pike, Bethesda, MD 20894, USA
FEATURES              Location/Qualifiers
  source              1..7215
                      /organism="Homo sapiens"
```

FIG.2A CONT.

```
5'UTR           782..790
CDS             join (791..970, 1237..1399, 2115..2264, 2499..2659,
                3207..3383, 4257..4398,4873..5060,5577..5718, 6308..6489)
                /gene=CYP2A6V2:
                /codon_start=1
                /product=cytochrome P450"
                /db_xref-PID:g1008462"
                /translation=MLASGMLLVALLACLTVMVLMSVWQQRKSKGKLPPGPTPLPFIG
                NYLQLNTEQMYNSLMKISERYGPVFTIHLGPRRVVVLCGHDAVREALVDQAEEFSGRG
                EQATFDWVFKGYGVVFSNGERAKQLLRFAIATLRDFGVGKRGIEERIQEESGFLIEAI
                RSTHGANIDPTFFLSRTVSNVISSIVFGDRFDYKDKEFLSLLRMMLGIFQFTSTSTGQ
                LYEMFSSVMKHLPGPQQQAFQLLQGLEDFIAKKVEHNQRTLDPNSPRDFIDSFLIRMQ
                EEEKNPNTEFYLKNLMMSTLNLFIAGTETVSTTLGYGFLLLMKHPEVEAKVHEEIDRV
                IGKNRQPKFEDRAKMPYMEAVIHEIQRFGDVIPMSLARRVKKDTKFRDFFLPKGIEVF
                PMLGSVLRDLRFFSNPRDFNPQHFLGEKGQFKKRDAFVPFSIRKRNCFGEGLARMELF
                LFFTTVMQNFRLKSSQSPKDIDVSPKHVGFATIPRNYTMSFLPR
exon            791..970
                /gene=CYP2A6V2:
                /number=1
exon            1237..1399
                /gene=CYP2A6V2:
                /number=2
exon            2115..2264
```

FIG.2A CONT.

```
                     /gene=CYP2A6V2:
                     /number=3
     exon            2499..2659
                     /gene=CYP2A6V2:
                     /number=4
     exon            3207..3383
                     /gene=CYP2A6V2:
                     /number=5
     exon            4256..4398
                     /gene=CYP2A6V2:
                     /number=6
     exon            4873..5060
                     /gene=CYP2A6V2:
                     /number=7
     exon            5577..5718
                     /gene=CYP2A6V2:
                     /number=8
     exon             6308..6489
                     /gene=CYP2A6V2:
                     /number=9
     3'UTR           6490..6744
BASE COUNT      1646 a    2196 c    1746 g    1627 t
ORIGIN
```

FIG.2A CONT.

BASE COUNT

1	aagttcccct	gaaatatggc	tctggtcttc	ctccccttgc	caatgaagaa	gatggcagtg
61	gaggttctat	ggcagccatc	ctggcctcac	tctgaggttc	caatgaggat	tctgggcatc
121	aagagacagc	tctgggcaaa	gctaaatcaa	gtcagcccct	ggacccagtg	ctgggctgct
181	gggctttctg	ggagaacgcc	gctgggcttg	ctacacactc	ctcctcccag	aaactccaca
241	cccacagccc	tgggtcttcc	tagccccgag	actttcaagt	ccatatgcct	ggaatccccc
301	ttcctgagac	ccttaaccct	gcatcctcca	caacagaaga	cccctaaatg	cacagccaca
361	ctttgtctta	ccctaataaa	acccagacct	ttggattcct	ctcccctgga	acccccagat
421	ccgcacaact	ttggggtgca	ttctcactct	cagaccccaa	atccaaagcc	caagtgctcc
481	cctatgcaaa	tattccaaac	tcctcagttc	tacagcttat	ctgttgcccc	ctcctaaatc
541	cacagccctg	cggcacccct	cctgaagtac	cacagattta	gtctggaggc	cccctctctg
601	ttcagctgcc	ctggggtccc	cttatcctcc	cttgctggct	gtgtcccaag	ctaggcagga
661	ttcatggtgg	ggcatgtagt	tgggaggtga	aatgaggtaa	ttatgtaatc	agccaaagtc
721	catccctctt	tttcaggcag	tataaaggca	aaccacccca	gccgtcacca	tctatcatcc
781	ctctaccacc	atgctggcct	cagggatgct	tctggtggcc	ttgctggcct	gcctgactgt
841	gatggtcttg	atgtctgttt	ggcagcagag	gaagagcaag	ggaagctgc	ctccgggacc
901	caccccattg	cccttcattg	gaaactacct	gcagctgaac	acagagcaga	tgtacaactc
961	cctcatgaag	gtgtcccaag	acagggagat	gggtgtctcg	gggtgggggc	tgcctagttg
1021	gctggggctt	tgtggcaggg	ggttgaccag	tgtggaccag	agtcttagga	aatggagttt
1081	tggagtttca	gcatcagaaa	gacaggatct	tgggatgtcc	agctccctga	ctgtgagaac
1141	ctgggtgcga	agcatcccag	cacatgacat	ctcggtgctg	ggccccattc	agagtggagg
1201	gttctccctc	taaccactcc	cacccacctc	catcagatca	gtgagcgcta	tggccccgtg
1261	ttcaccattc	acttggggcc	ccggcgggtc	gtggtgctgt	gtggacatga	tgccgtcagg

FIG.2A CONT.

```
1321 gaggctctgg tggaccaggc tgaggagttc agcgggcgag gcgagcaagc caccttcgac
1381 tgggtcttca aaggctatgg tgcccaagag ggggaaggtg ggcaggtgga cacgaaggtc
1441 tcagtgttcc cagccttctc cctgactctc ctgacaactg gaggataagg gagagtcccc
1501 agtctggtct tccctcccca tctccctaca ttggggcctc tccatgtgta tccctcacct
1561 gtctccagcg gccctgtcct gattcctccc tgcctctctc tgccccacct ccttattctc
1621 tctcactgga gtctcctctt tcccctctct tcccctctct ctccatctct tgggtttctg
1681 tttaccagcc ctgggtctct gtctacatga gtctttgagg ccctcttagc ttctgggctt
1741 ctctgggttt ctcatctctc cggatccctt tctcaattct tcctctgtct taggatgcca
1801 gggttattcc tacttccaca tcttcaggct ccatctcctg gtaacagtct ctcttccttc
1861 cagaccctct ctgtttctat ctcaatatta aactctctgc tccagctcag cttaagaatc
1921 tcacaccaag agaggatgtc ctccacccag atctccccat atctcactac cccaccctcc
1981 atcctctgcc tccatcactc tctttctctc cccactgccc tgcggacgcg atccaatgga
2041 gtgtggagct aatgccgtga agctatgtgc atctctctgt ctggccgtac ctgggtaata
2101 acctgatcga ctaggcgtgg tattcagcaa cggggagcgc gccaagcagc tcctgcgctt
2161 tgccatcgcc accctgaggg acttcggggt gggcaagcga ggcatcgagg agcgcatcca
2221 ggaggagtcg ggcttcctca tcgaggccat ccggagcacg cacggtgagc aggggacccc
2281 gagtgcgggg gcaggagaag gaaaacaccc aggacgagga acccgcgcgc gttctgcctg
2341 gggatgggga ctaggtgggg aaaggcgccc gcacttccag ccctggagtc tggcgctggg
2401 aatttggctc aacaaggccc tgcctcctgg aattctgact ctcctcagac ctctgagttg
2461 actctctccc caacccccctt ctcccgacat acccggaggc gccaatatcg atcccacctt
2521 cttcctgagc cgcacagtct ccaatgtcat cagctccatt gtctttgggg accgctttga
2581 ctataaggac aaagagttcc tgtcactgtt gcgcatgatg ctaggaatct tccagttcac
2641 gtcaacctcc acggggcagg taatggttgc agcccggccc gtgaaggccc ttaccaaaac
```

FIG.2A CONT.

```
2701 cggcaaattg ttcccctacc gggggaaggg ggccccaaat tcccaccgcc ccccggacag
2761 tgtcccctca aaatcagtcc ccgatttggg caaattggca gagtggaacc agacccgggt
2821 tggttgtcca atcccctgct ctccagggac accgggatag cacaacagat gctccccaaa
2881 acagagcctg ctggcaggat gcataccctc agctcagctc tctcaccctg ggcacgtgtt
2941 cccatcccca acttaccggt aatttctaac agatgctccc tacccaggtc ttcttgaata
3001 ttttaacacc cggaaaccct gggtacctaa ccttcctgt  aaactttaga gattagttcc
3061 tatccggccc ctctgaaata cctaaccacc ggagaccaga tgcctttaac tcagttcctt
3121 ccttgctatg aaacaaatcc cattcccatc agctcctgcc ccgtgacagc tgtccttccc
3181 ttcccatcct ctctctgcaa ccccagctct atgagatgtt ctcttcggtg atgaaacacc
3241 tgccaggacc gcagcaacag gcctttcagt tgctgcaagg gctggaggac ttcatagcca
3301 agaaggtgga gcacaaccag cgcacgctgg atcccaattc cccacgggac ttcattgact
3361 cctttctcat ccgcatgcag gaggtacacc ccagcagcca ctgcggggag atgcaaagcc
3241 aggcagaggg aaatcagtct gggagtgggg caggcagatg acacaggccc attcaaatta
3481 accctcatca taataatcct cacaattggc tgggtgccgt ggctaacagc ctgtaatccc
3541 agcactttgg gaggccgagg caggtggatc acctgaggtc aggagttcga gaccagcctg
3601 gccaacatgg tcaaaccccg tctctactaa aaatccaaaa attagttggg catggtggcg
3661 cgaagggggg cagaggttgc aatgagccaa gatcacggca ttgcactcca gtctgggtga
3721 cagaatgagg ccctgtgtca aaaaaaatta atcacttgtt taaaaagtaa gtgagcctgc
3781 atggtcatgc gcatgtgcag ctccagctac tcaggaggct gaggctggag gattgcttga
3841 gctcaggagt tggcgtccgg cctgtgcaac ttagcaagac caagtcagta taagaaaaaa
3901 aaaaaacaaa aaaaaagctg acagctaagt tgataattga cggacagatg gtcagcaagg
3961 taacgaaggt gagaaggaag agcattgggg gcaacgccag gagtcagggc aagggctggt
```

FIG.2A CONT.

```
4021 tcctagagcg agtctggtag gatctagggc ccctcttctc caccctgcgg tcttgcccca
4081 aagagaggtc gagggtgctg ggattgcgct agactcgagt ctgtgtagat cttggggtcc
4141 cctcttgacc cccattggtc tgaacctaag agtggaagat ccatggggtg aacccctaga
4201 tggtgccctg aggtcaagca ggagtgaggt tgtcctaaag ccccctctcc cttcaggagg
4261 agaagaaccc caacacggag ttctacttga agaacctgat gatgagcacg ttgaacctct
4321 tcattgcagg caccgagacg gtcagcacca ccctgcacta tggcttctta ctgctcatga
4381 agcacccaga ggtggagggt aaggctggag ggggacggaa gtggagggcc ccagaccctc
4441 aaaattcccc ttcgactggt gcaatgtccc cacctgtccc agatcccggg accctgagac
4501 gtgacttgct gtccagagac agggcaacat tcagctggta ggcatcagct gagtctcatt
4561 agatattaaa atattgaaaa tgtctgcact gattggtcag tcacttctgt cccaagccca
4621 ctgagtgccc actgcccgtt ccaccgggtc atcccctaag ttcctccctg tgcctccct
4681 gtgattctgg cacaacctgg ttaacaggat cctactccaa caatgcgaat gggtgatgtc
4741 tgttctgtta tgaatgctct acttccgtct cataggcgga ggcatttcat ccaccccatt
4801 ttgcctatcc ggactatcat ttcctgctct gagaccccta gatacctaaa cacattcccc
4861 ctcctccccc agccaaggtc catgaggaga ttgacagagt gatcggcaag aaccggcagc
4921 ccaagtttga ggaccgggcc aagatgccct acatggaggc agtgatccac gagatccaaa
4981 gatttggaga cgtgatcccc atgagtttgg cccgcagagt caaaaaggac accaagtttc
5041 gggatttctt cctccctaag gtgctatccg cccccacccc ccagactacg gggactccag
5101 cccctctctg tgtccccagc atcccaccca cattagaagc tttctagacc ctgtcccact
5161 ccctcaatca gtcaaaaaag acttccccaa ccaccacatc cgttccacct ttccacttag
5221 acactcctga gtcctgcatc tctccagact ctttgtgtca ggagaatcaa acacatgttc
5281 ccaaacttcc tatcttaaga aacagaagcc ccctttccat tcggcctttt gtcataggga
5341 cagaaatctc aggtccccca aactcctgcc tagaaggaca tggaccccat gtctcccaaa
```

FIG.2A CONT.

```
5401 cttcctgttt cagagatgtg aaccttctat cccccaaggt cctccctcag aggtccccaa
5461 ttcccatgcc tgccacttcc cctcaccggg gcaccctagt tccccctcca gcccctgtgt
5521 actctcaaca atcccccaac ccgcctcatc acatacacct tcctcctccc tcccagggca
5581 tagaagtgtt ccctatgttg ggctccgtgc tgagagacct caggttcttc tccaaccccc
5641 gggacttcaa tccccagcac ttcctgggtg agaaggggca gtttaagaag cgtgatgctt
5701 ttgtgccctt ctccatcagt aagagaccac tgtttggtgc caggcttact actcacacca
5761 gcaggggcct cccttaccca gttcccctct ctgccgtgta gcctagtatt tccccagctt
5821 ggcaagttcc tgttagcaat ctaccgtcga gccaccaggt gatactccct taactaccaa
5881 gcacccagta cctgtgccca ggcaaaagga aaggaaacat catacccctt tcagaggcgg
5941 gggaaaacca aaggccagag agaatcagag atttatttcc ctagggtcac acaggagatt
6001 cttcagcatc cctaaaaagg agatgacggc acagcaggtc atatttggga gttcttatct
6061 gggggaaggg ggatcttaaa cctcccattg tggacacctg gcatcgatca accccatctt
6121 ttggtcatct tttgggtcac tcaaggaaac tgaggtcaag gagggtcaag aggctccctc
6181 ttaaagtctc tcagggccat atattccacc cttcctccct gggagagccg cagctggagg
6241 tcggtactgg ggcgaggctg cactgagagt gggcttcacc tccacccctc ccgcctctcc
6301 tcctcaggaa agcggaactg tttcggagaa ggcctggcca gaatggagct ctttctcttc
6361 ttcaccaccg tcatgcagaa cttccgcctc aagtcctccc agtcacctaa ggacattgac
6421 gtgtccccca aacacgtggg ctttgccacg atcccacgaa actacaccat gagcttcctg
6481 ccccgctgag cgagggctgt gccggtgaag gtctggtggg cggggccagg gaaagggcag
6541 ggccaagacc gggcttggga gaggggcgca gctaagactg ggggcaggat ggcggaaagg
6601 aaggggcgtg gtggctagag ggaagagaag aaacagaagc ggctcagttc accttgataa
6661 ggtgcttccg agctgggatg agaggaagga aacccttaca ttatgctatg aagagtagta
```

FIG.2A CONT.

```
6721 ataatagcag ctcttatttc ctgagcacgt acccccgtgt cacctttgtt caaaaaccat
6781 tgcacgctca cctaatttgc cacaaaaccc ccttcgaagg ggcgttcatg cccattttac
6841 acgtgacaaa actgaggctt agaaagttgt ctctgatgtc tcacaaaaca taagtgccca
6901 gaaaatctgc gaacacagat ctgtgcccat agccttctag acagattctt aaaaagcacc
6961 tattcctcac gcaaaacagt ttagtataga atcacatggc ctgaacatcc ctgtccgggg
7021 gagttcccca gagacctggg gggtggttgc cctgccttca ctgcacacat gcccacactc
7081 tcacctactc aacatgctgt gactacccgg gtgtaatctg tgcttgctac cagataaggc
7141 cactgtagcc cattcagagt cagcccaggg acacaacgag acatgactgg acatacaggg
7201 tcagtccatt aacaa
```

FIG.2B

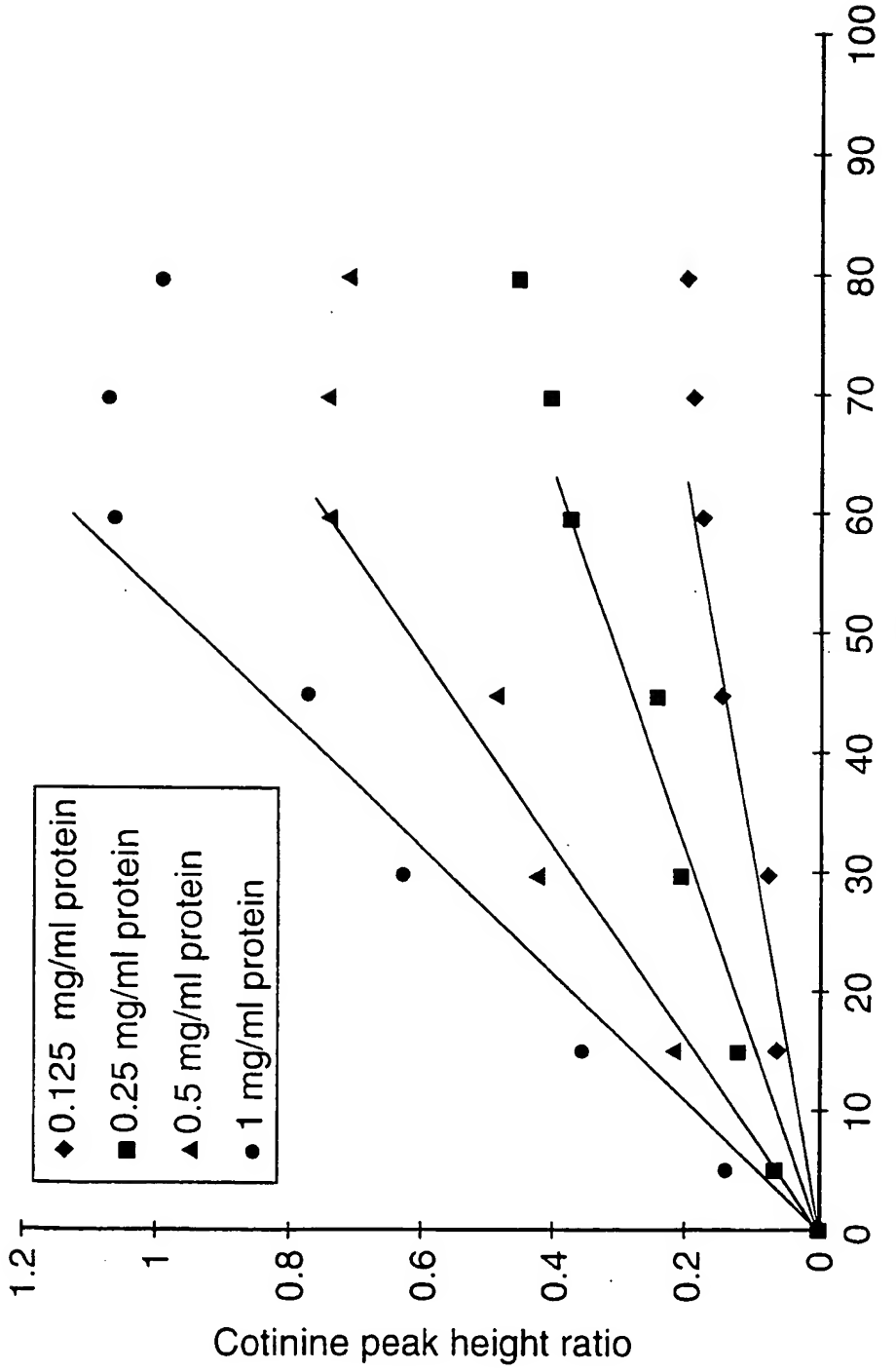
```
LOCUS       HSP452B6     1415  bp   RNA          PRI          29-MAY-1992
DEFINITION  Human MRNA FOR CYTOCHROME P-450IIVB6.
ACCESSION   X13494
NID         g35206
KEYWORDS    Cytochrome; cytochrome P450IIB6.
SOURCE      human.
  ORGANISM  Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 1415)
  AUTHORS   Miles, J.S.
TITLE       Direct Submission
  JOURNAL   Submitted (10-NOV-1988) Miles J.S., Imperial Cancer Research Fund,
            Lab of Molecular Phrmacology and Drug Metabolism, Hugh Robson
            Building, George Square, Edinburgh, EH8 9XD
REFERENCE   2 (bases 1 to 1415)
  AUTHORS   Miles, J.S., McLaren, A.Q. and Wolf, C.R.
  TITLE     Alternative splicing in the human cytochrome P450IIB6 gene
            generates a high level of aberrant messages
  JOURNAL   Nucleic Acids Res. 17 (20), 8241-8255 (1989)
  MEDLINE   90045947
COMMENT     The sequence is a compilation of genomic and cDNA clones. **map:
            chromosomal location=19q12-13.2;
            Data kindly reviewed (13-NOV-1989) by Miles, J.S.
FEATURES             Location/Qualifiers
```

FIG.2B CONT.

```
     source          1..1415
                     /organism="Homo sapiens"
      misc_feature   9..110
                     /note=exon 1, partial"
      misc_feature   111..273
                     /note=exon 2"
       misc_feature  274..423
                     /note=exon 3"
       misc_feature  424..584
                     /note=exon 4"
      misc_feature   585..761
                     /note=exon 5"
      misc_feature   762..903
                     /note=exon 6"
      misc_feature   904..1091
                     /note=exon 7"
      misc_feature   1092..1233
                     /note=exon 8"
       misc_feature  1234..1415
                     /note=exon 9", coding region"
BASE COUNT      341 a    430 c    328 g    316 t
ORIGIN
        1 gaattccgcc ctgcacccat gaccgcctcc caccagggcc ccgccctctg  ccccttttgg
```

FIG.2B CONT.

```
  61 gaaaccttct gcagatggat agaagaggcc tactcaaatc ctttctgagg ttccgagaga
 121 aatatgggga cgtcttcacg gtacacctgg gacccaggcc cgtggtcatg ctgtgtggag
 181 tagaggccat acgggaggcc cttgtggaca aggctgaggc cttctctggc cggggaaaaa
 241 tcgccatggt cgacccattc ttccggggat atggtgtgat ctttgccaat ggaaaccgct
 301 ggaaggtgct tcggcgattc tctgtgacca ctatgaggga cttcgggatg ggaaagcgga
 361 gtgtggagga gcggattcag gaggaggctc agtgtctgat agaggagctt cggaaatcca
 421 agggggccct catggacccc accttcctct tccagtccat taccgccaac atcatctgct
 481 ccatcgtctt tggaaaacga ttccactacc aagatcaaga gttcctgaag atgctgaact
 541 tgttctacca gacttttca ctcatcagct ctgtattcgg ccagctgttt gagctcttct
 601 ctggcttctt gaaatacttt cctggggcac acaggcaagt ttacaaaaac ctgcaggaaa
 661 tcaatgctta cattggccac agtgtggaga agcaccgtga aaccctggac cccagcgccc
 721 ccaaggacct catcgacacc tacctgctcc acatggaaaa agagaaatcc aacgcacaca
 781 gtgaattcag ccaccagaac ctcaacctca acacgctctc gctcttcttt gctggcactg
 841 agaccaccag caccactctc cgctacggct tcctgctcat gctcaaataccctcatgttg
 901 cagagagagt ctacagggag attgaacagg tgattggccc acatcgccct ccagagcttc
 961 atgaccgagc caaaatgcca tacacagagg cagtcatcta tgagattcag agattttccg
1021 accttctccc catgggtgtg ccccacattg tcacccaaca caccagcttc cgagggtaca
1081 tcatccccaa ggacacagaa gtatttctca tcctgagcac tgctctccat gacccacact
1141 actttgaaaa accagacgcc ttcaatcctg accactttct ggatgccaat ggggcactga
1201 aaaagactga agcttttatc cccttctcct tagggaagcg gatttgtctt ggtgaaggca
1261 tcgcccgagc ggaattgttc ctcttcttca ccaccatcct ccagaacttc tccatggcca
1321 gccccgtggc cccagaagac atcgatctga caccccagga gtgtggtgtg ggcaaaatac
1381 ccccaacata ccagatccgc ttcctgcccc gctga
```



Protein-time curves of cotinine production from 100 µM nicotine in the presence of 20 µl rat cytosol by K20 human liver microsomes.

FIG.3

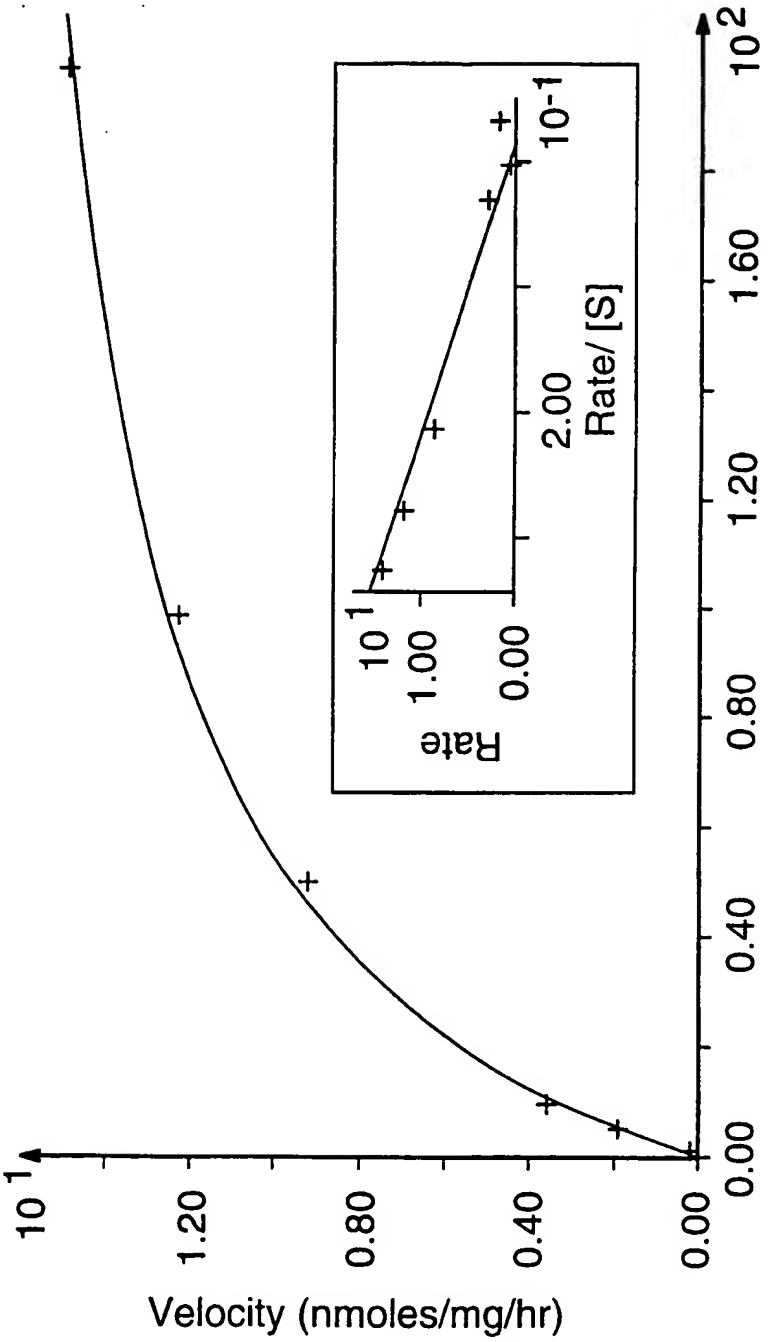

Velocity (nmoles/mg/hr)
10 1
1.20
0.80
0.40
0.00
Nicotine (uM)
0.00
0.40
0.80
1.20
1.60
10 2
Rate
10 1
1.00
0.00
Rate/ [S]
2.00
10-1

FIG.4A

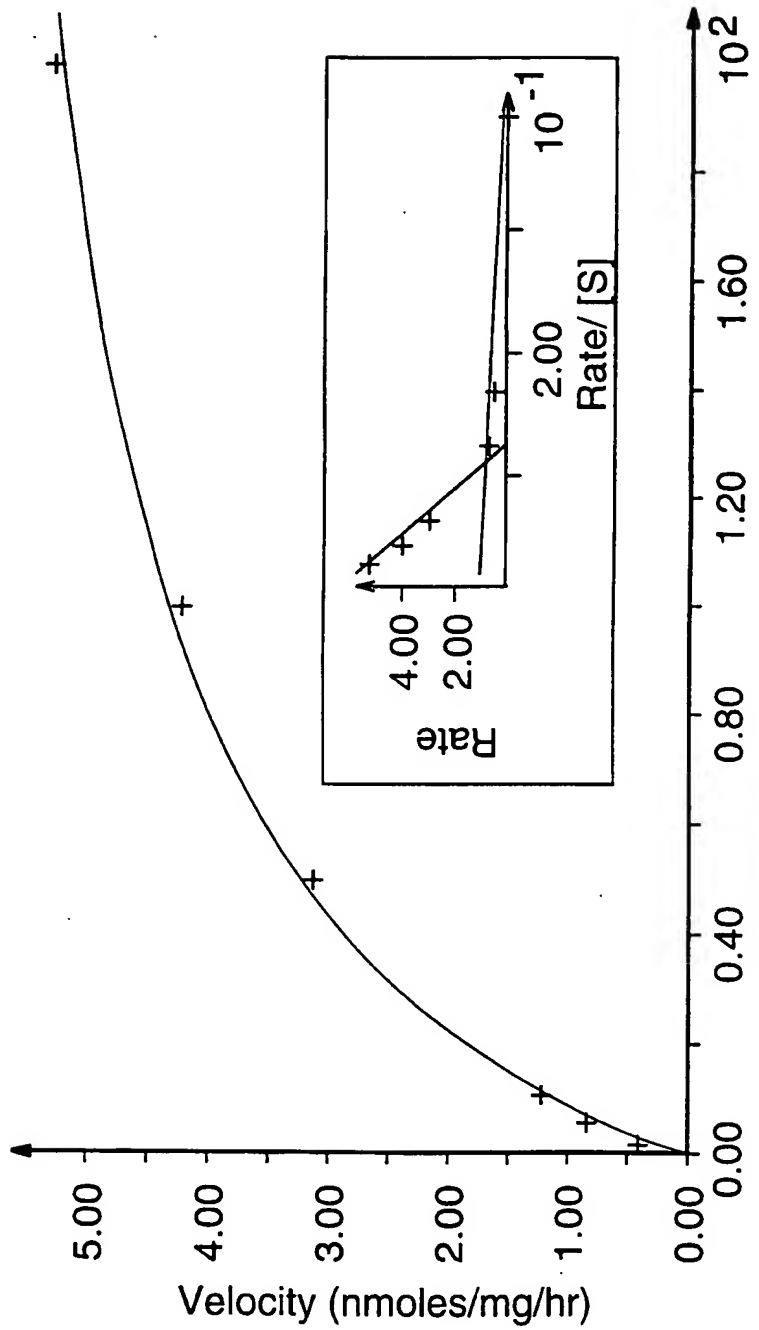

Velocity (nmoles/mg/hr)
5.00
4.00
3.00
2.00
1.00
0.00
Nicotine (uM)
0.00
0.40
0.80
1.20
1.60
10 2
Rate
4.00
2.00
Rate/ [S]
2.00
10 -1

FIG.4B

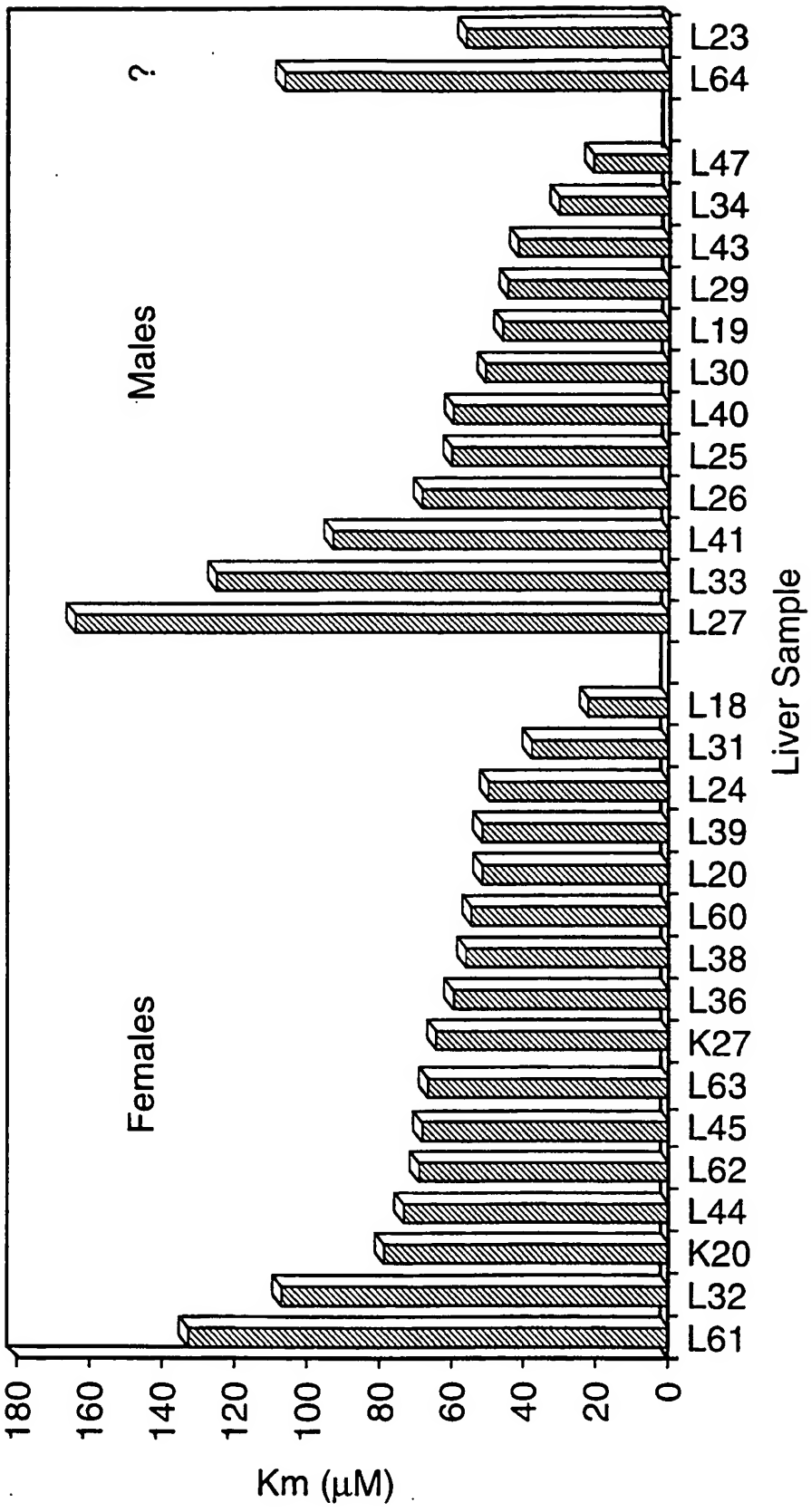

?
Males
Females
L23
L64
L47
L34
L43
L29
L19
L30
L40
L25
L26
L41
L33
L27
L18
L31
L24
L39
L20
L60
L38
L36
K27
L63
L45
L62
L44
K20
L32
L61
180
160
140
120
100
80
60
40
20
0
Km (µM)
Liver Sample

FIG.5

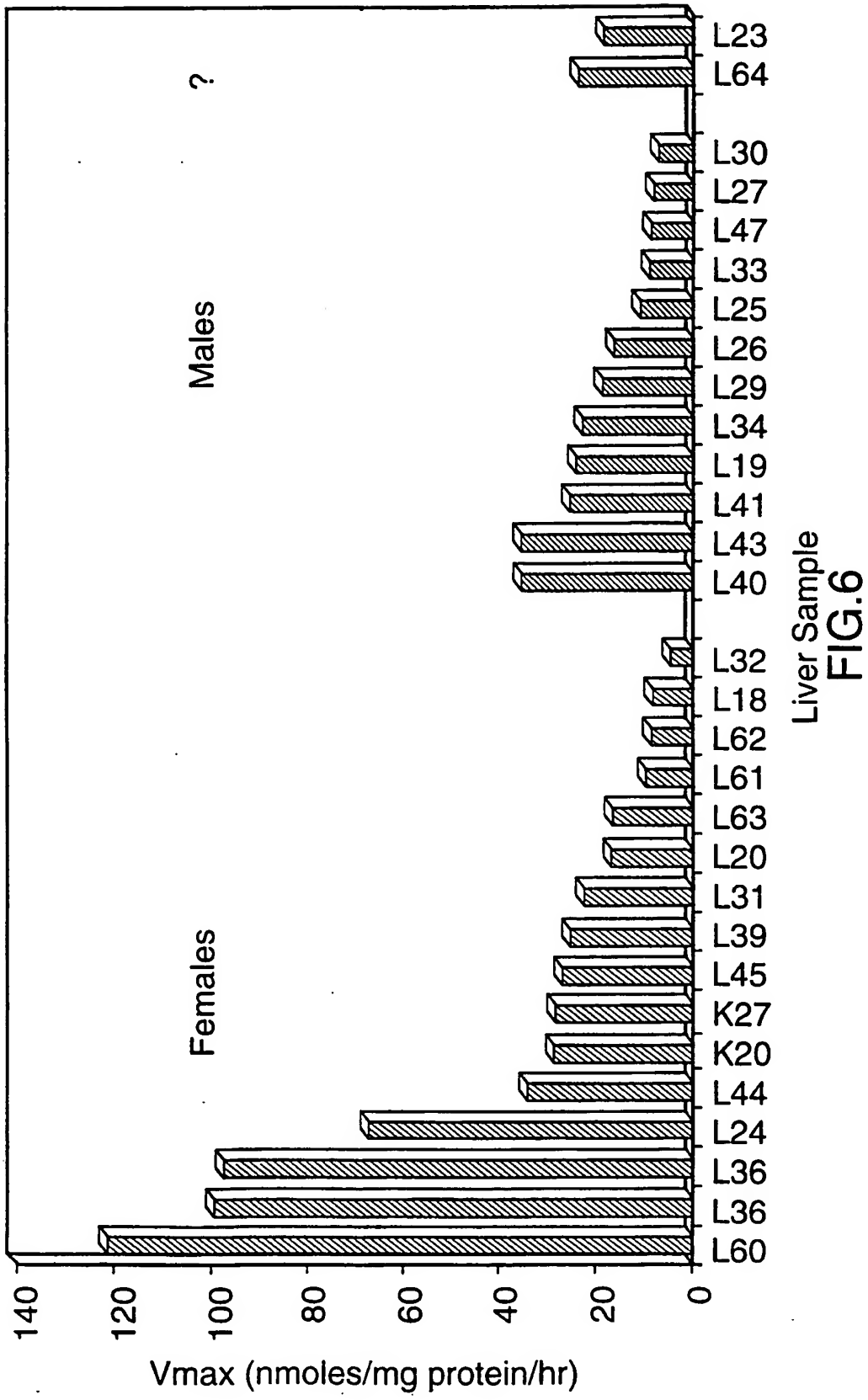

?
Males
Females
L23
L64
L30
L27
L47
L33
L25
L26
L29
L34
L19
L41
L43
L40
L32
L18
L62
L61
L63
L20
L31
L39
L45
K27
K20
L44
L24
L36
L36
L60
Liver Sample
140
120
100
80
60
40
20
0
Vmax (nmoles/mg protein/hr)

FIG.6

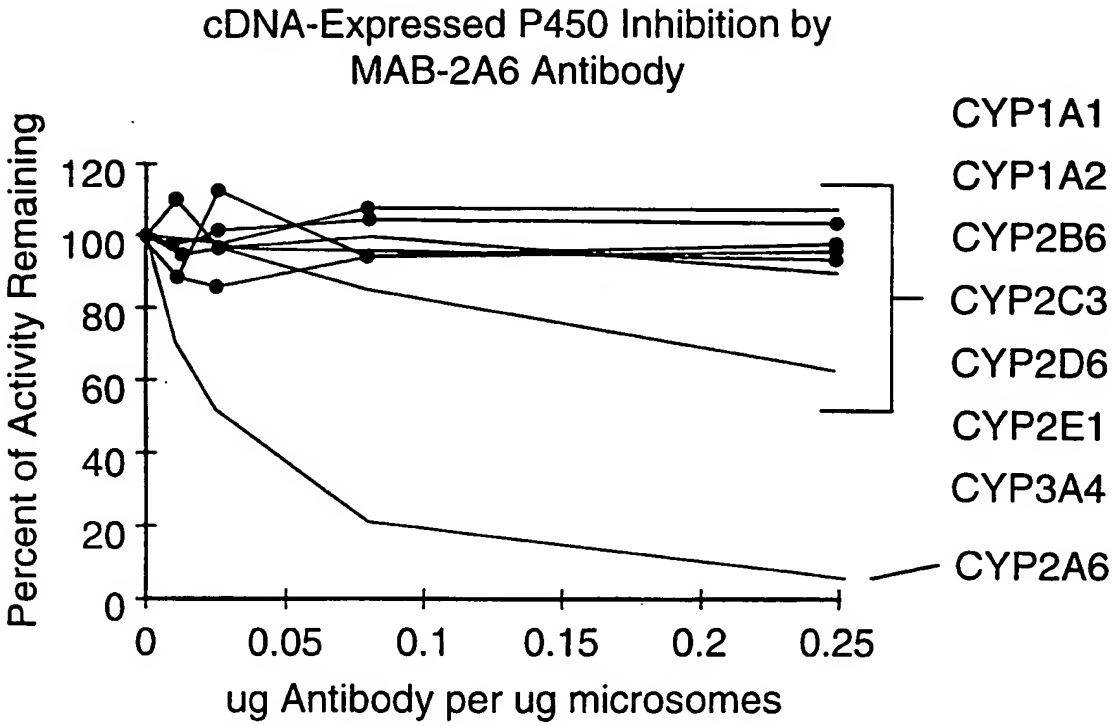

cDNA-Expressed P450 Inhibition by
MAB-2A6 Antibody
Percent of Activity Remaining
120
100
80
60
40
20
0
0
0.05
0.1
0.15
0.2
0.25
ug Antibody per ug microsomes
CYP1A1
CYP1A2
CYP2B6
CYP2C3
CYP2D6
CYP2E1
CYP3A4
CYP2A6

FIG.7A

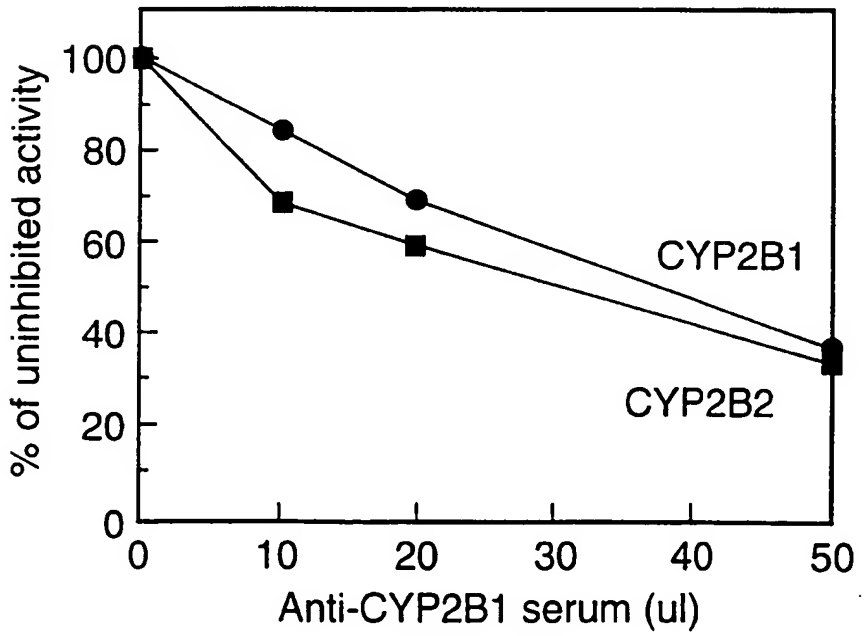

% of uninhibited activity
100
80
60
40
20
0
0
10
20
30
40
50
Anti-CYP2B1 serum (ul)
CYP2B1
CYP2B2

FIG.7B

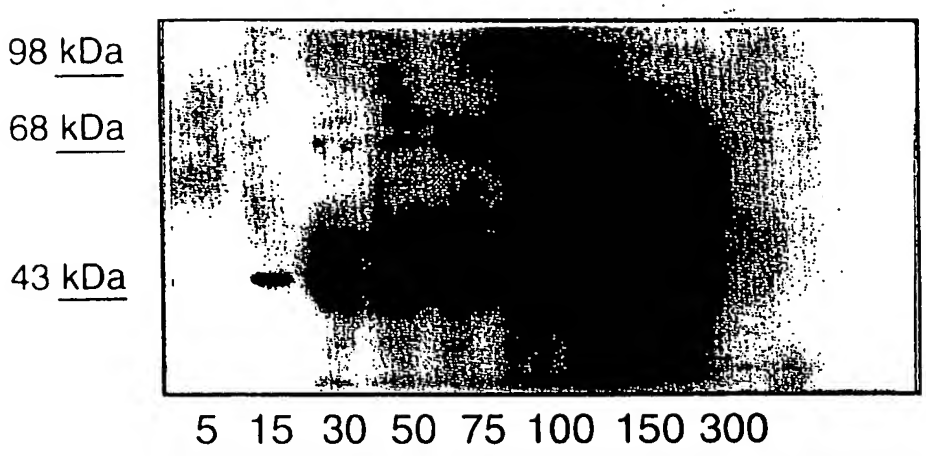


FIG.8A

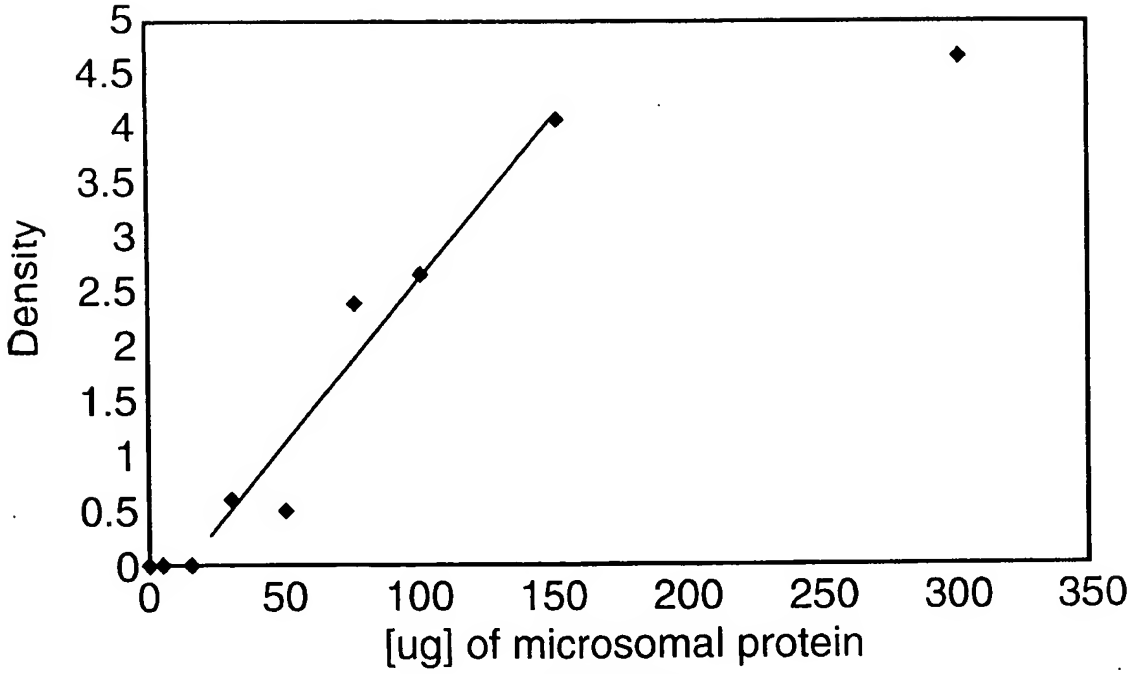


FIG.8B

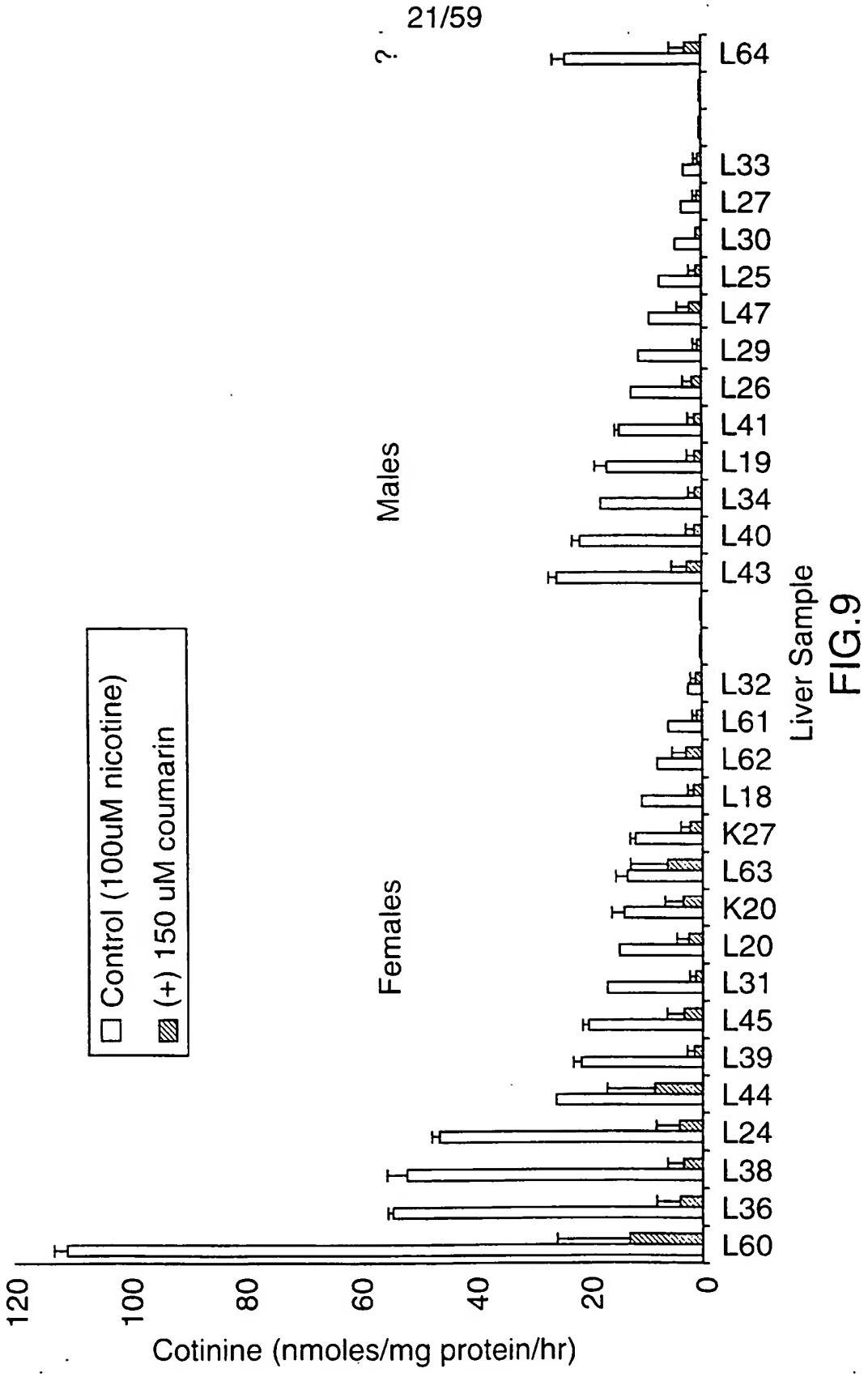

Control (100uM nicotine)
(+) 150 uM coumarin
Males
Females
L64
L33
L27
L30
L25
L47
L29
L26
L41
L19
L34
L40
L43
L32
L61
L62
L18
K27
L63
K20
L20
L31
L45
L39
L44
L24
L38
L36
L60
Liver Sample
120
100
80
60
40
20
0
Cotinine (nmoles/mg protein/hr)

FIG.9

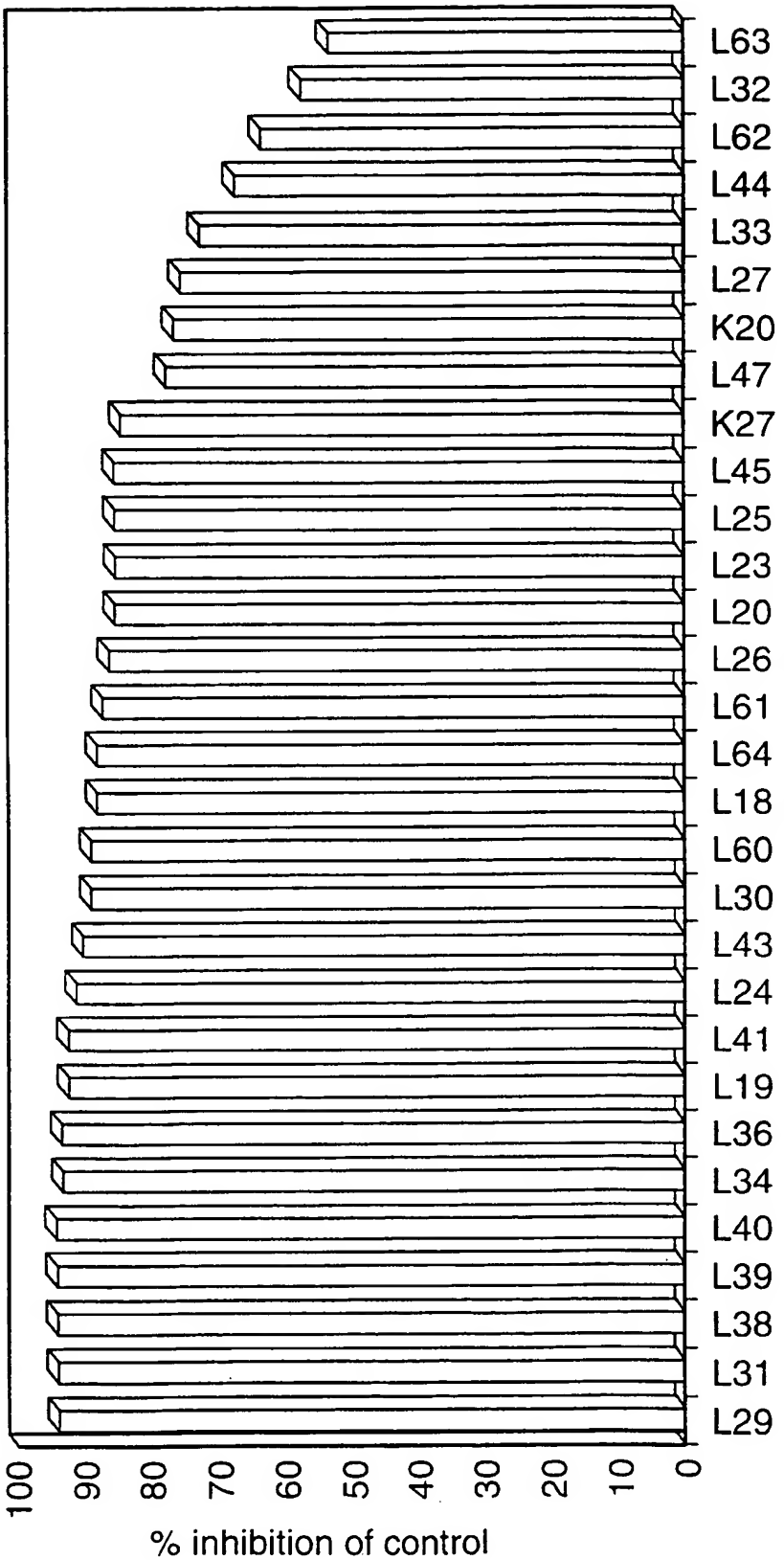

L63
L32
L62
L44
L33
L27
K20
L47
K27
L45
L25
L23
L20
L26
L61
L64
L18
L60
L30
L43
L24
L41
L19
L36
L34
L40
L39
L38
L31
L29
100
90
80
70
60
50
40
30
20
10
0
% inhibition of control
Liver Sample

FIG.10

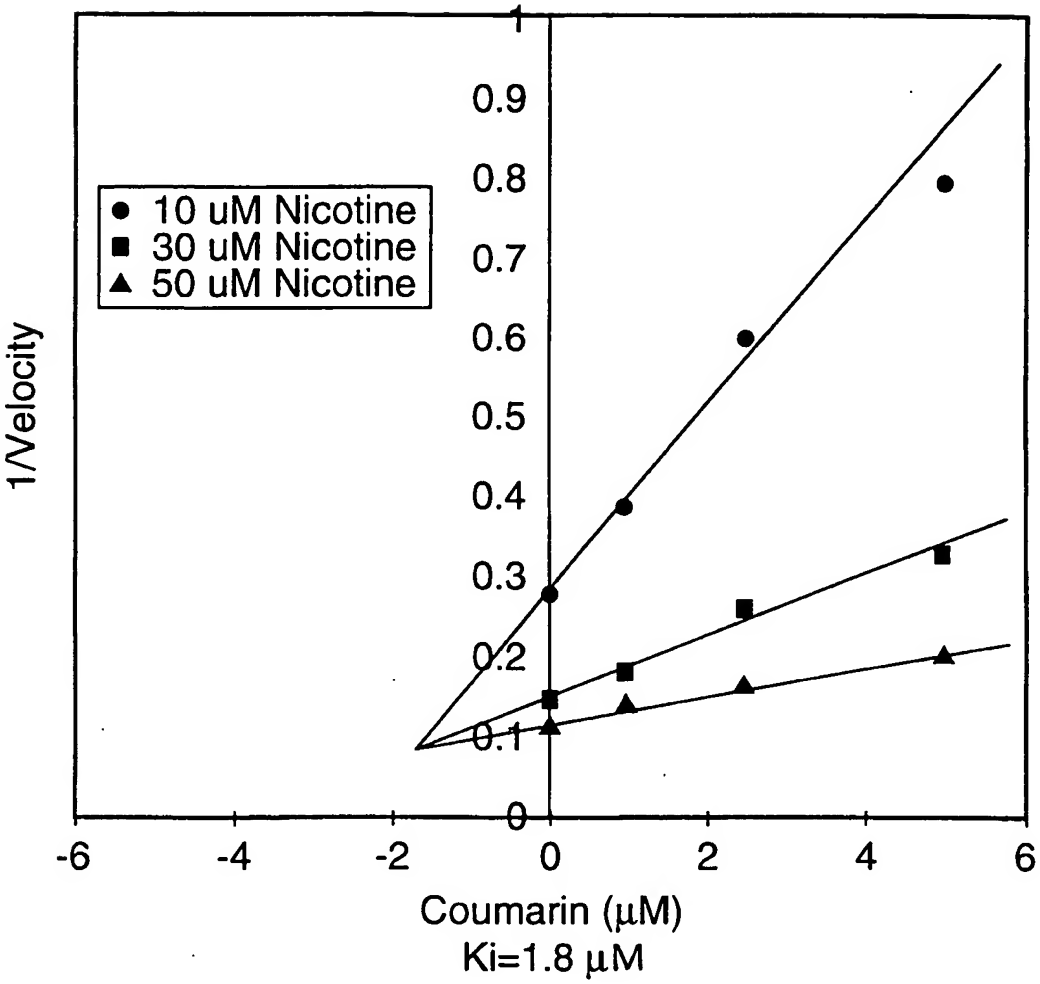

1/Velocity
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-6
-4
-2
0
2
4
6
10 uM Nicotine
30 uM Nicotine
50 uM Nicotine
Coumarin (μM)
Ki=1.8 μM

FIG.11

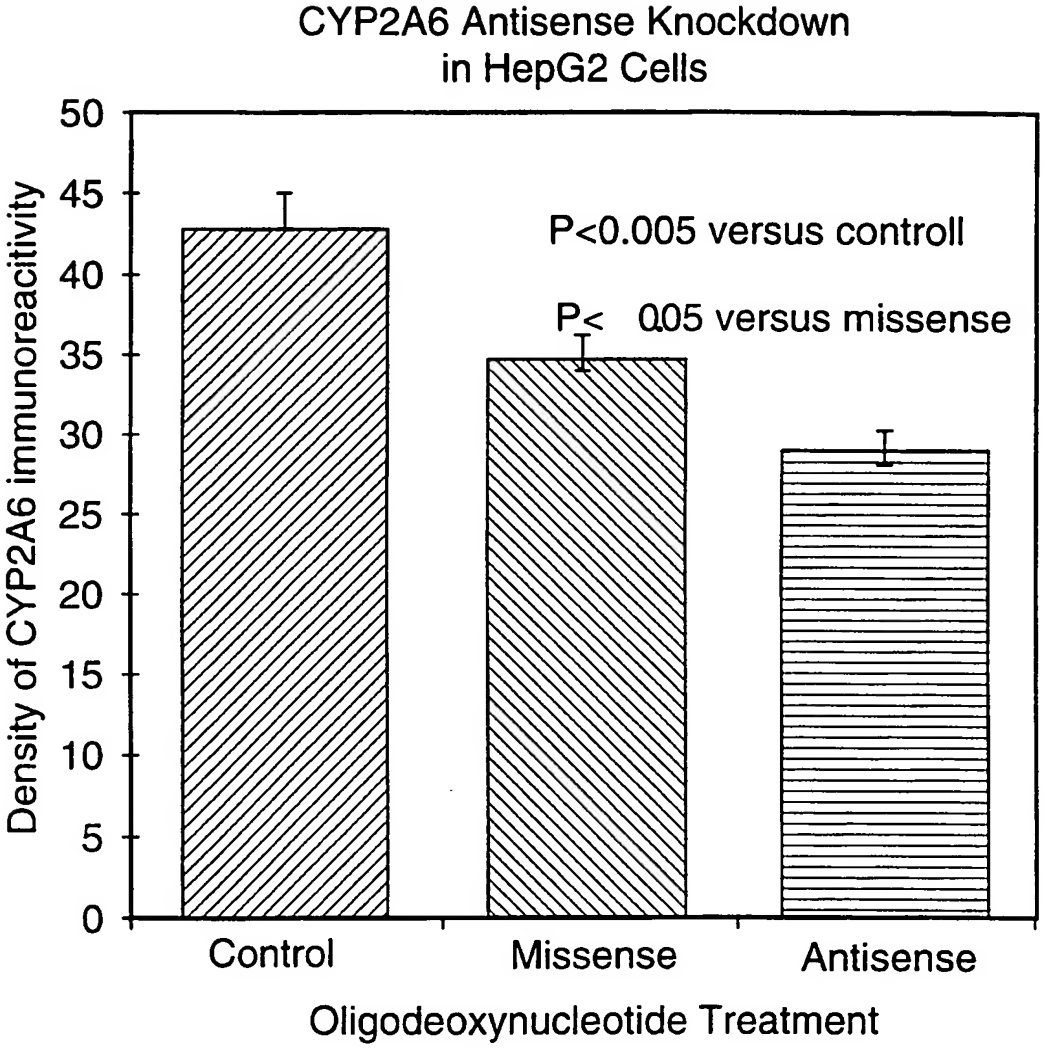

CYP2A6 Antisense Knockdown
in HepG2 Cells
Density of CYP2A6 immunoreactivity
50
45
40
35
30
25
20
15
10
5
0
P<0.005 versus controll
P< 0.05 versus missense
Control
Missense
Antisense
Oligodeoxynucleotide Treatment

FIG.12

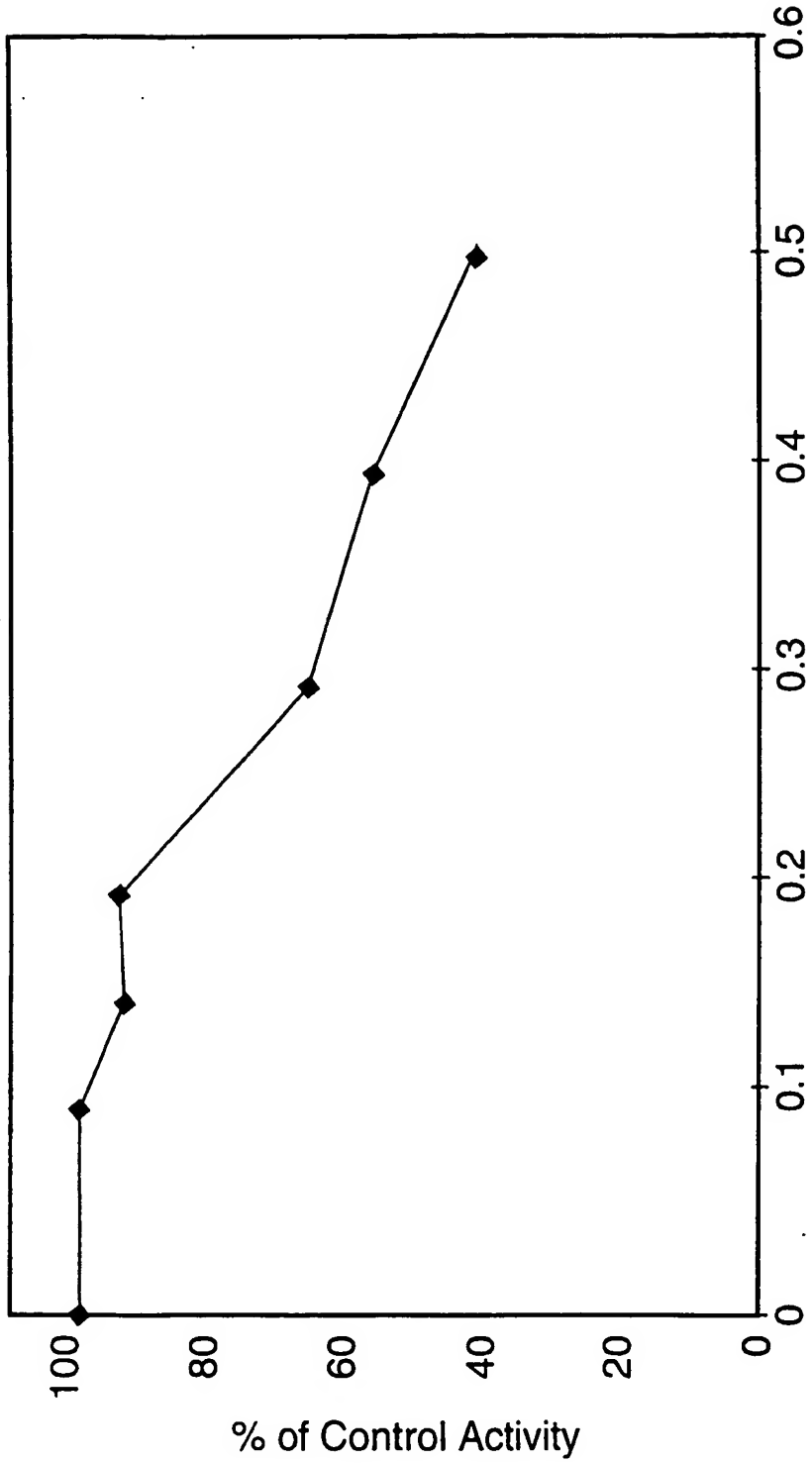
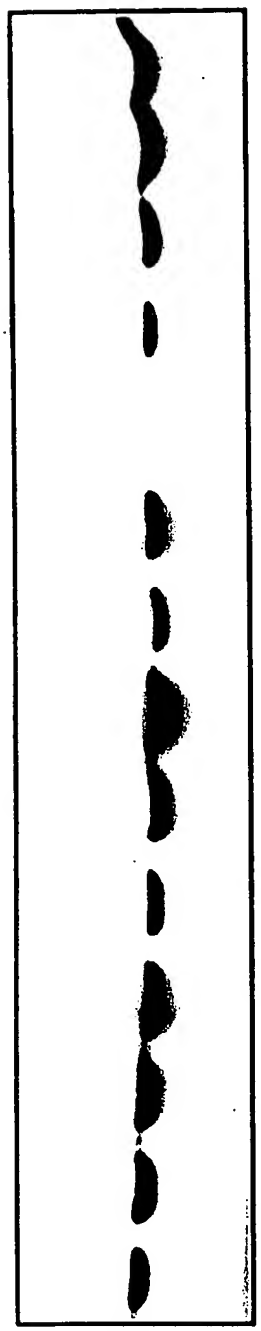
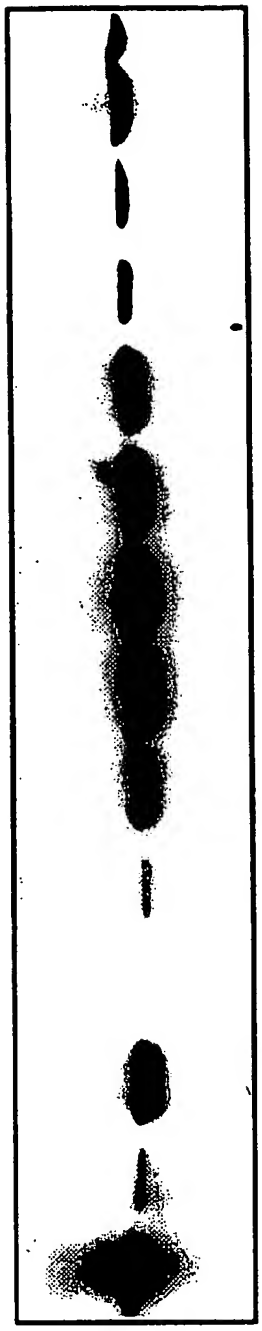

0.6
0.5
0.4
0.3
0.2
0.1
0
µg Antibody/µg MS protein
100
80
60
40
20
0
% of Control Activity

FIG.13



K20 K27 L18 L19 L20 L23 L24 L25 L26 L27 15 30 75 100

FIG.14A.



L29 L30 L31 L32 L33 L34 L36 L38 L39 L40 15 30 75 100

FIG.14B.

L41 L43 L44 L45 L47 L60 L61 L62 L63 L64 15 30 75 100

FIG.14C.

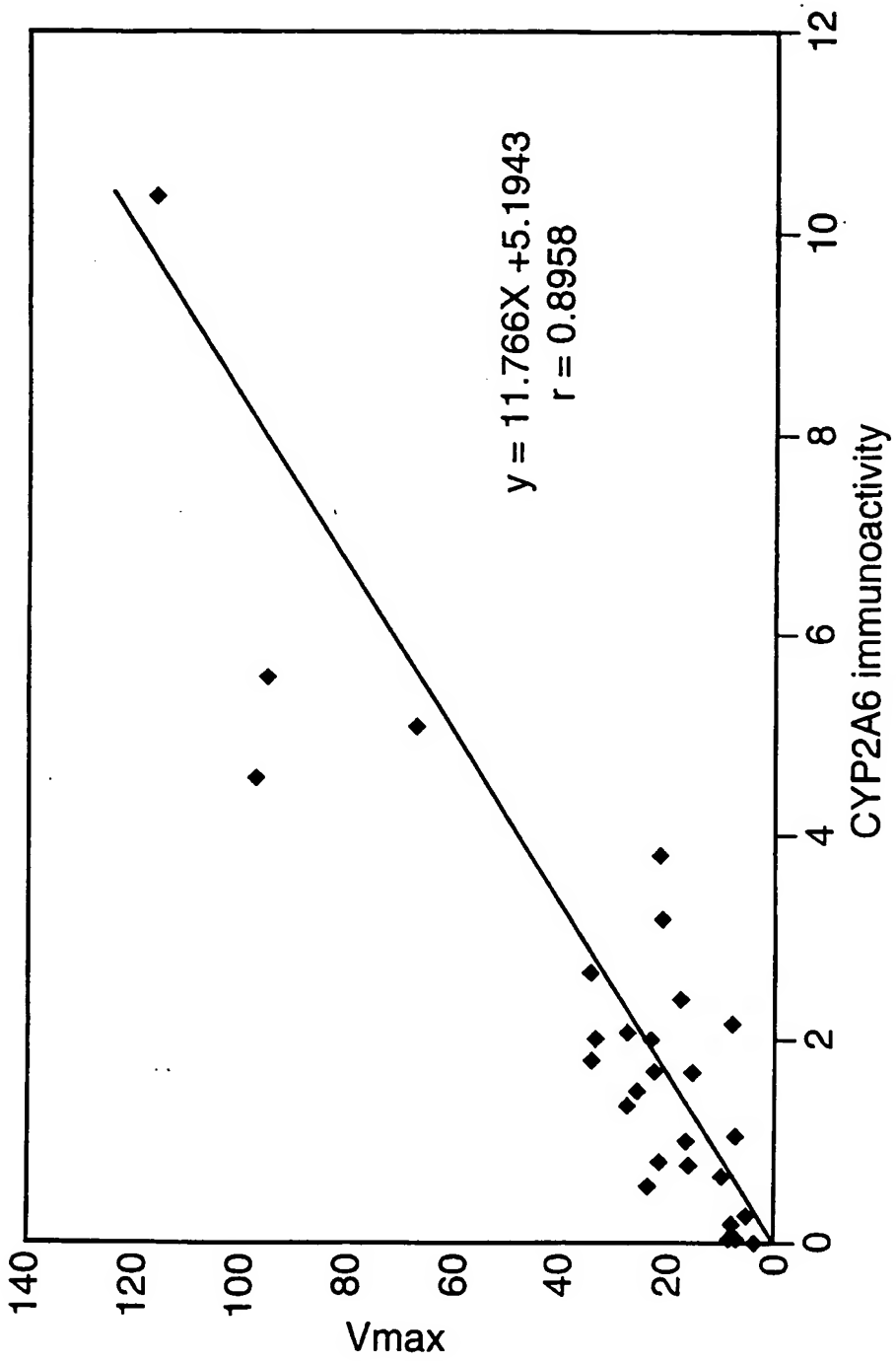

y = 11.766X +5.1943
r = 0.8958
CYP2A6 immunoactivity
Vmax
0
2
4
6
8
10
12
0
20
40
60
80
100
120
140

FIG.15

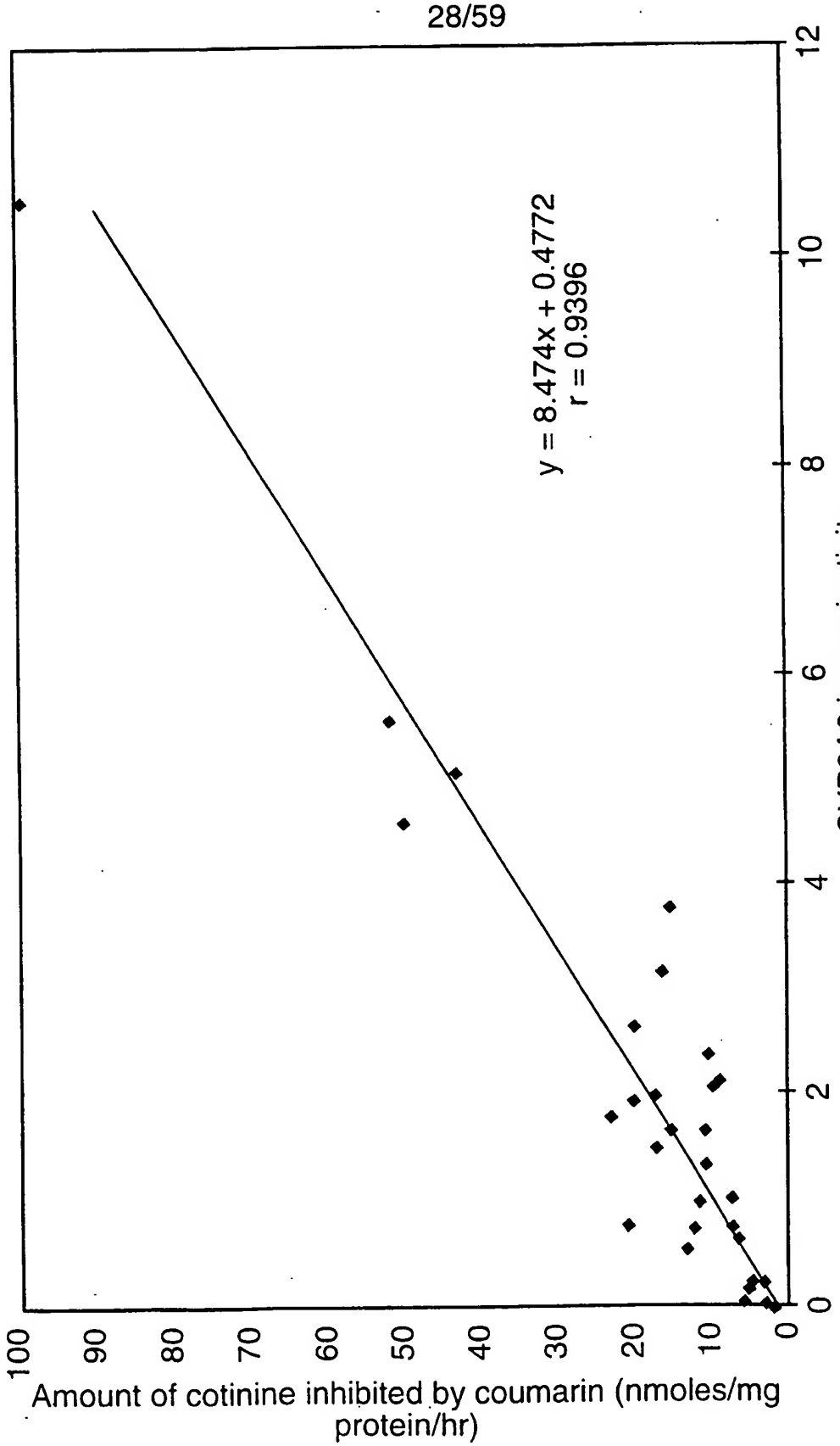

y = 8.474x + 0.4772
r = 0.9396
CYP2A6 immuniactivity
0
2
4
6
8
10
12
100
90
80
70
60
50
40
30
20
10
0
Amount of cotinine inhibited by coumarin (nmoles/mg protein/hr)

FIG.16

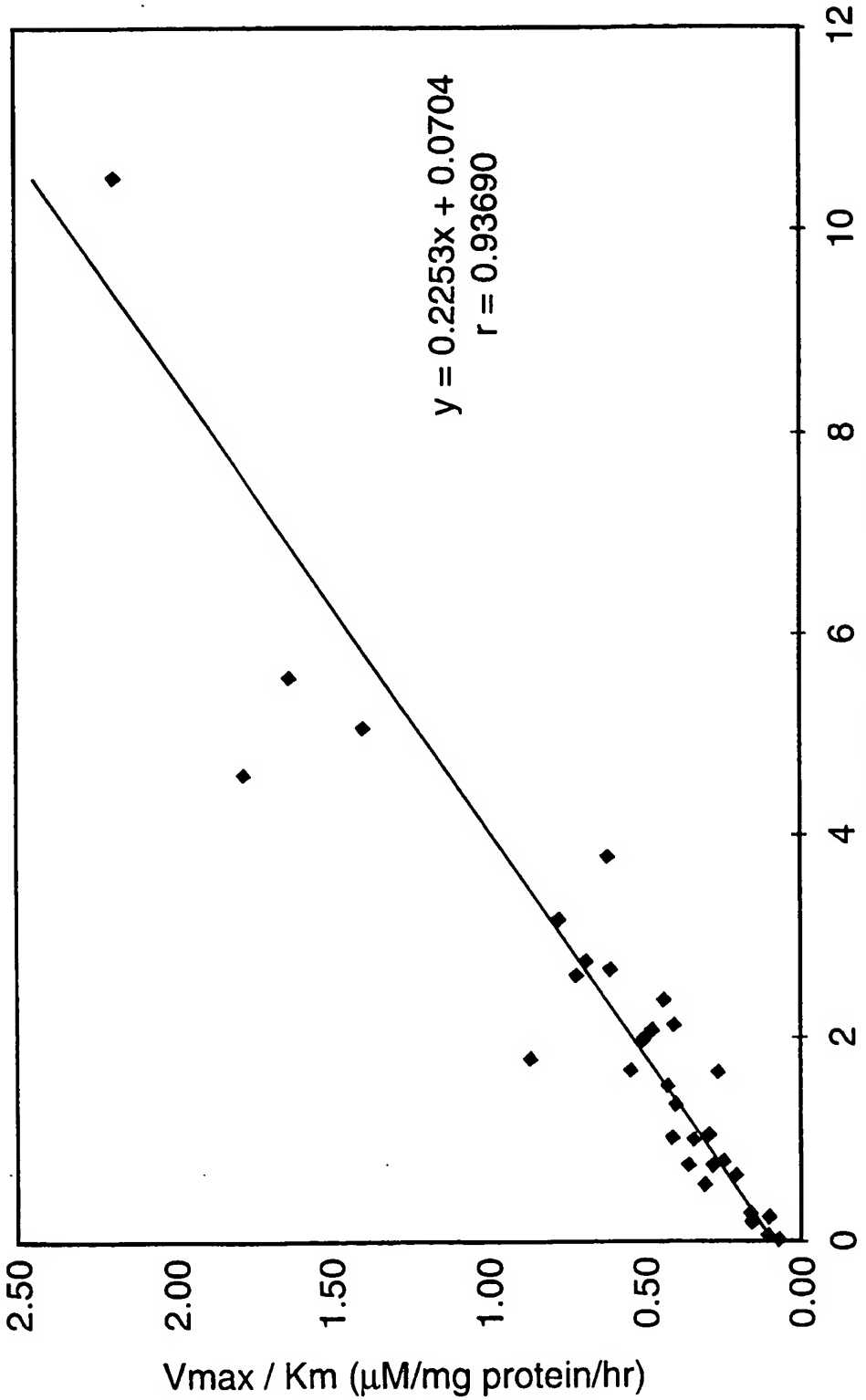

y = 0.2253x + 0.0704
r = 0.93690
CYP2A6 immunoactivity
0
2
4
6
8
10
12
Vmax / Km (µM/mg protein/hr)
0.00
0.50
1.00
1.50
2.00
2.50

FIG.17

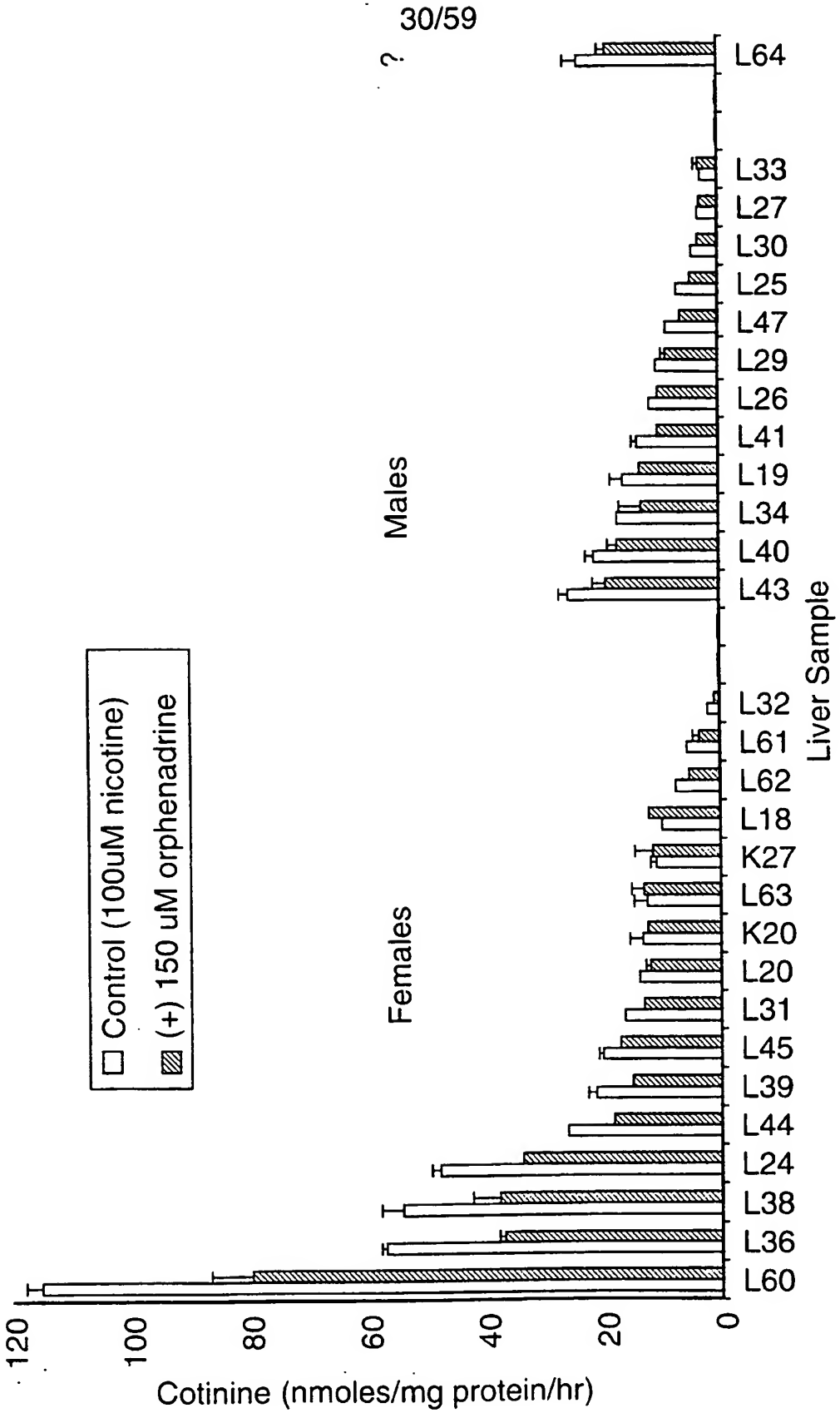

Control (100uM nicotine)
(+) 150 uM orphenadrine
Males
Females
L64
L33
L27
L30
L25
L47
L29
L26
L41
L19
L34
L40
L43
L32
L61
L62
L18
K27
L63
K20
L20
L31
L45
L39
L44
L24
L38
L36
L60
Liver Sample
120
100
80
60
40
20
0
Cotinine (nmoles/mg protein/hr)

FIG.18

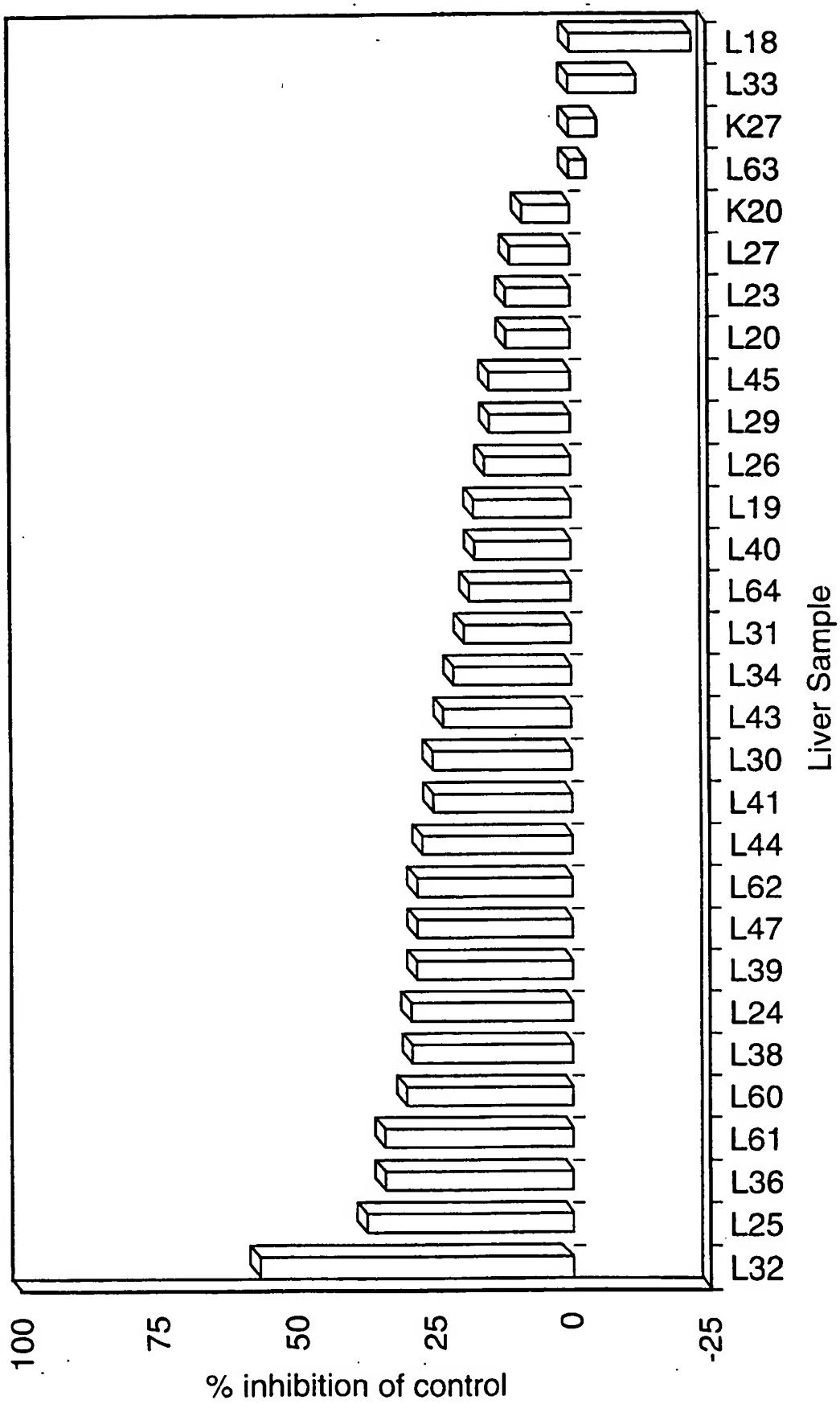

L18
L33
K27
L63
K20
L27
L23
L20
L45
L29
L26
L19
L40
L64
L31
L34
L43
L30
L41
L44
L62
L47
L39
L24
L38
L60
L61
L36
L25
L32
Liver Sample
100
75
50
25
0
-25
% inhibition of control

FIG.19

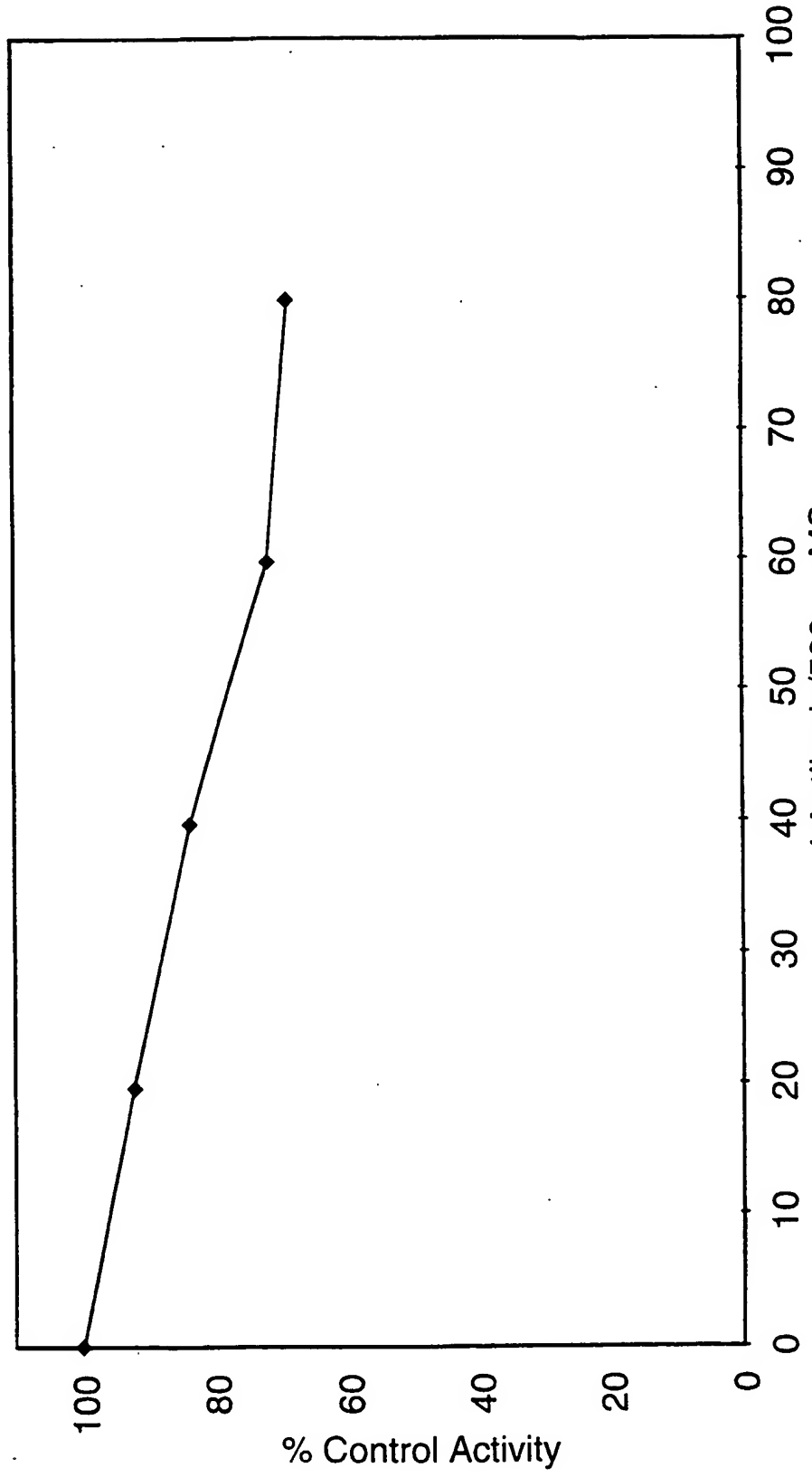

100
80
60
40
20
0
% Control Activity
0
10
20
30
40
50
60
70
80
90
100
µl Antibody/500 µg MS

FIG.20

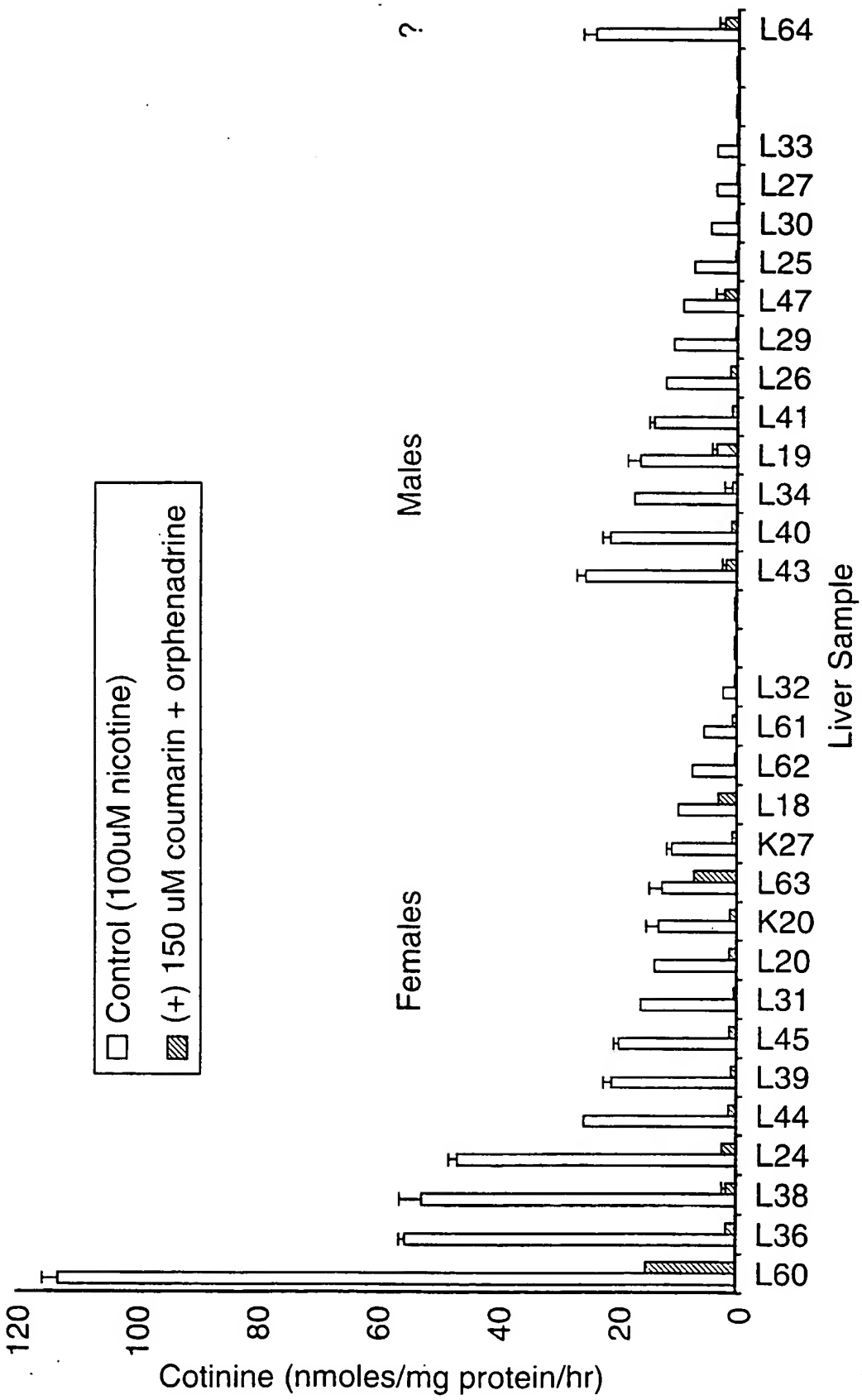

Control (100uM nicotine)
(+) 150 uM coumarin + orphenadrine
Males
Females
?
L64
L33
L27
L30
L25
L47
L29
L26
L41
L19
L34
L40
L43
L32
L61
L62
L18
K27
L63
K20
L20
L31
L45
L39
L44
L24
L38
L36
L60
Liver Sample
120
100
80
60
40
20
0
Cotinine (nmoles/mg protein/hr)

FIG.21

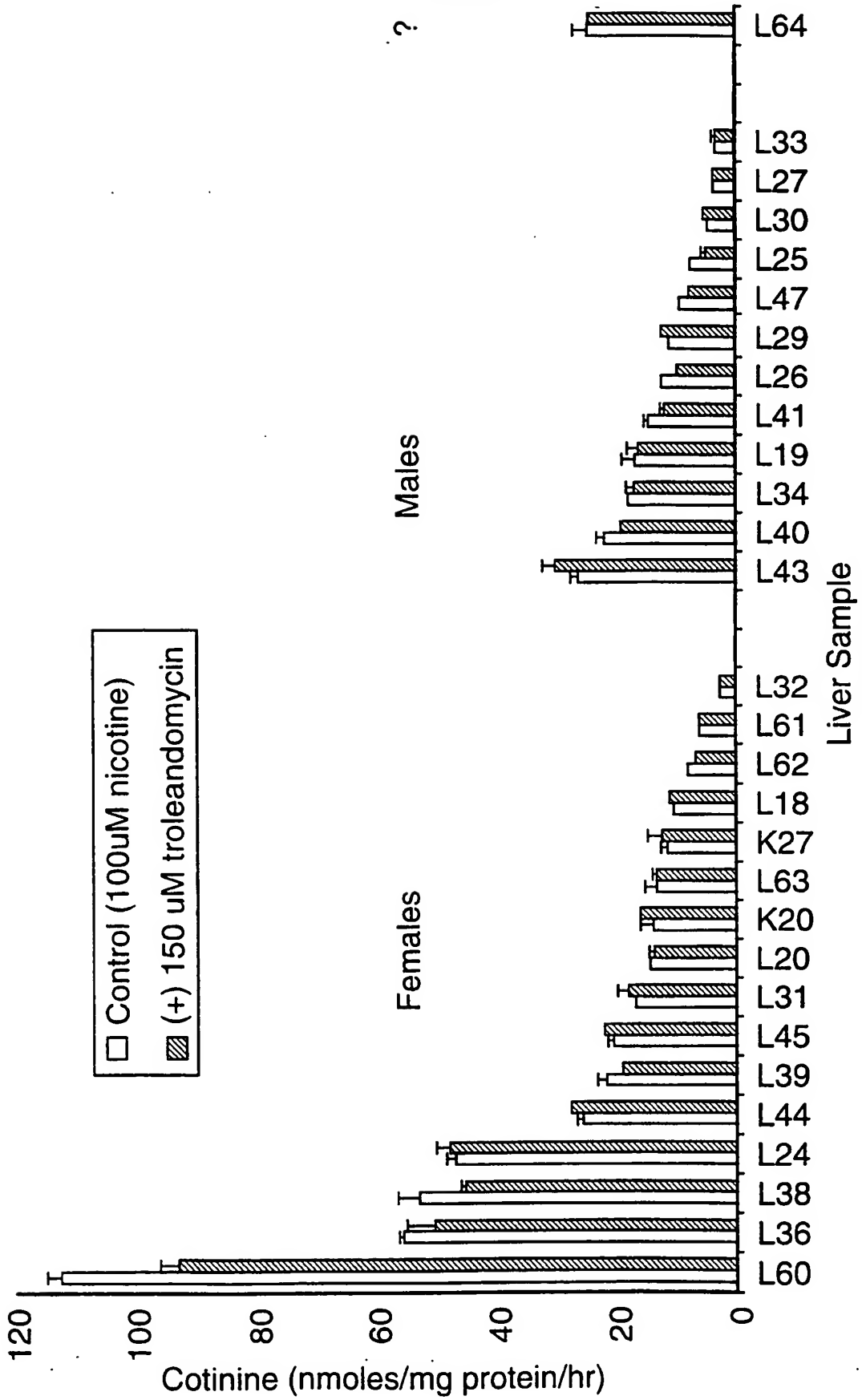
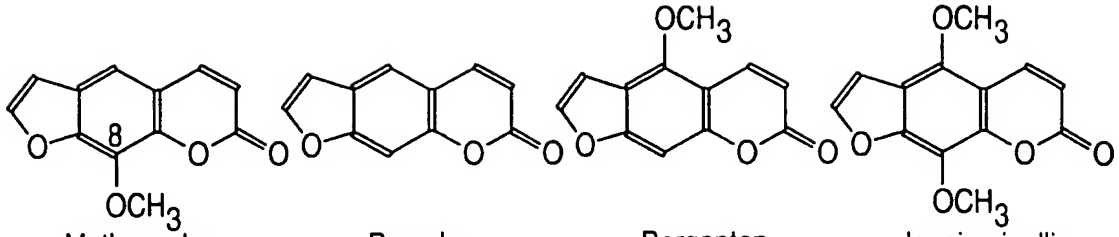
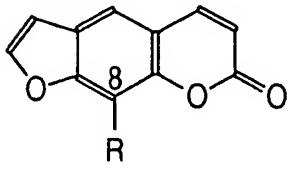

Control (100uM nicotine)
(+) 150 uM troleandomycin
Cotinine (nmoles/mg protein/hr)
120
100
80
60
40
20
0
Females
Males
Liver Sample
L60
L36
L38
L24
L44
L39
L45
L31
L20
K20
L63
K27
L18
L62
L61
L32
L43
L40
L34
L19
L41
L26
L29
L47
L25
L30
L27
L33
L64

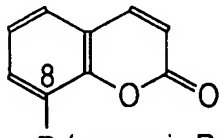
FIG.22



Methoxsalen ic50 5.4 | Psoralen IC50 110 | Bergapten IC50>500 | Isopimpinellin IC50>500

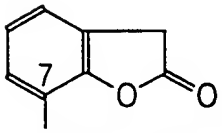


R= $-OCH_2CH_3$ $-CH_2CH$ $-OH$ $-C_6H_5$
$-OCH_2CH_2CH_3$ $-CH_2CH_2CH_3$ $-NH_2$
$-OCH_2CH_2CH_4CH_3$ $-CH_2CH_2CH_2CH_3$ $-NO_2$



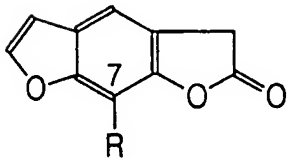
R (coumarin R-H)

R= $-OCH_3$ $-CH_3$
$-OCH_2CH_3$ $-CH_2CH_3$ $-OH$ $-C_6H_5$
$-OCH_2CH_2CH_3$ $-CH_2CH_2CH_3$ $-NH_2$
$-OCH_2CH_2CH_2CH_3$ $-CH_2CH_2CH_2CH_3$ $-NO_2$

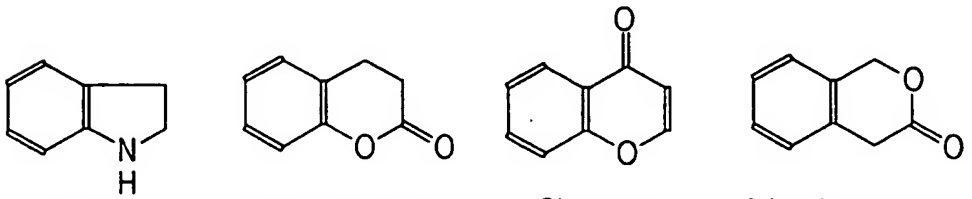


R (2-coumaranone R=H)

R= -H
$-OCH_3$ $-CH_3$ $-OH$ $-C_6H_5$
$-OCH_2CH_3$ $-CH_2CH_3$ $-NH_2$
$-OCH_2CH_2CH_3$ $-CH_2CH_2CH_3$ $-NO_2$
$-OCH_2CH_2CH_2CH_3$ $-CH_2CH_2CH_2CH_3$

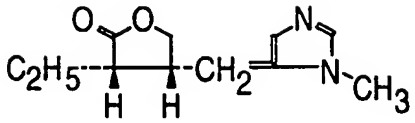


R= $-OCH_3$ (7-methocy -4',5',-5',6-coumaranone)
$-CH_3$ $-OH$ $-C_6H_5$
$-OCH_2CH_3$ $-CH_2CH_3$ $-NH_2$
$-OCH_2CH_2CH_3$ $-CH_2CH_2CH_3$ $-NO_2$
$-OCH_2CH_2CH_2CH_3$ $-CH_2CH_2CH_2CH_3$

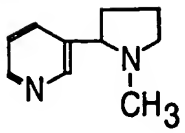


Indole | Dihydrocoumarin | Chomone | 3-Isochromanone

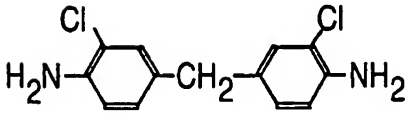
FIG.23A



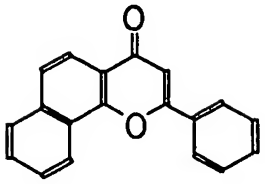
Pilocarpine



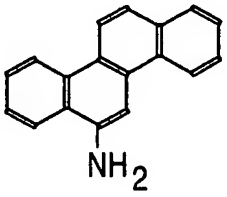
Nicotine



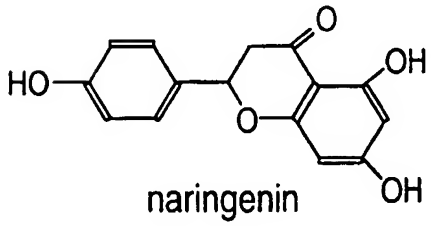
4,4'-Methylene bis[2-chloroaniline



6-Aminochrysene



α-Naphthoflavone



naringenin

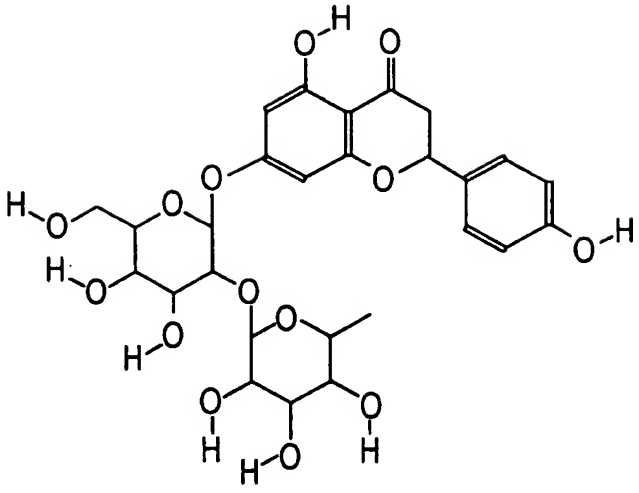
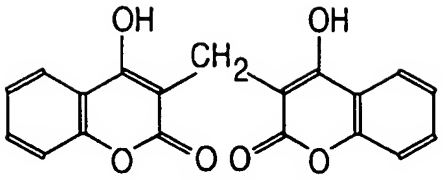
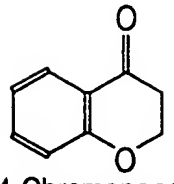


FIG.23B

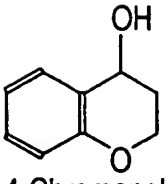


Dicumarol

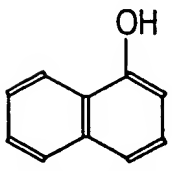
About 80% activity left at 0.05 mM concentration



4-Chromanone

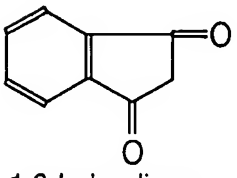


4-Chromanol

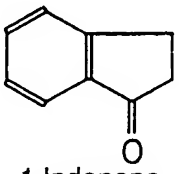


1-Naphthol

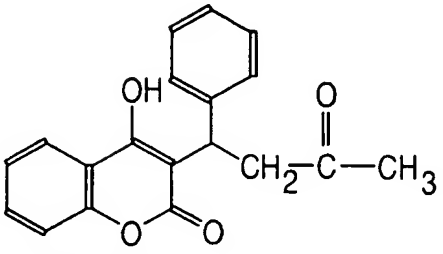
70% inhibition at
0.5 mM concentration



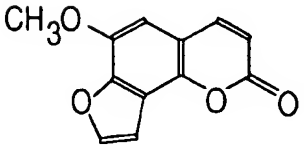
1,3-Indandione



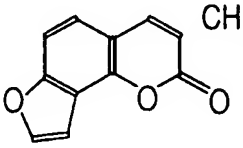
1,Indandone



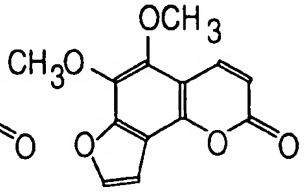
Warfarin



Sphondin
IC50 90



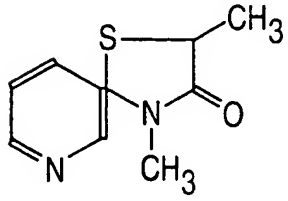
Amgelicin
IC 50 160



Pimpinrllin
IC50>500

$(CH_3CH_2)_2NCS_2NH_4$

Diethyldithiocarbamic acid
ammonium salt



SM-12502

$[(CH_3)_2N]_3P(O)$

Hexamethylphosphoramide

$(CH_3)_2NNO$

N-Nitrosodimethylamine

FIG.23C

FIG.24A

The SAS System
Experiment BC1; Pharmacokinetics of nicotine
Revised analysis of kinetics based on re-assays
Does treatment affect AUC?

---------- Compound assayed=COTININE ----------

Dependent Variable: AUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	10578731978	151124745	4.66	0.0397
Error	6	1944298022	324049670		
Corrected Total	13	12523030000			

R-Square	C.V.	Root MSE	AUC Mean
0.844742	19.80871	18001.38	90876.07

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SUBJ	6	10548143898	1758023983	5.43	0.0294
TREATMNT	1	30588081	30588081	0.09	0.7690

Least Squares Means

TREATMNT	AUC LSMEAN
Methoxsalen10-50	92354.2010
Placebo	89397.9447

FIG.24B

The SAS System
Experiment BC1; Pharmacokinetics of nicotine
Revised analysis of kinetics based on re-assays
Does treatment affect AUC?

---------- Compound assayed=NICOTINE ----------

Dependent Variable: AUC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	54879492.87	7839927.55	5.14	0.0317
Error	6	9143654.02	1523942.34		
Corrected Total	13	64023146.88			

R-Square	C.V.	Root MSE	AUC Mean
0.857182	17.22829	1234.481	7165.426

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SUBJ	6	23085554.55	3847592.43	2.52	0.1422
TREATMNT	1	31793938.32	31793938.32	20.86	0.0038

Least Squares Means

TREATMNT	AUC LSMEAN
Methoxsalen10-50	8672.40779
Placebo	5658.44323

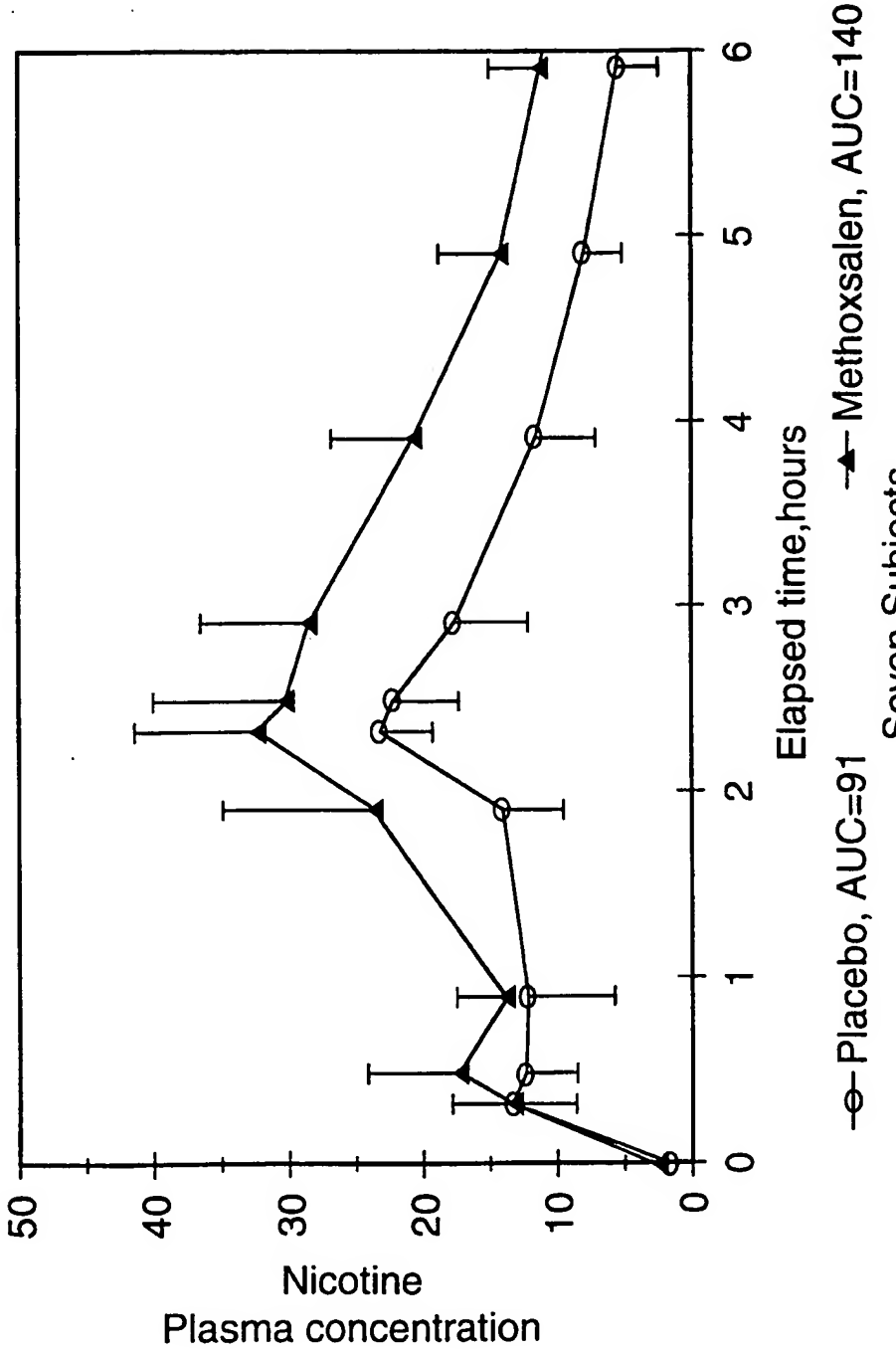

Nicotine
Plasma concentration
50
40
30
20
10
0
0
1
2
3
4
5
6
Elapsed time,hours
Placebo, AUC=91
Methoxsalen, AUC=140
Seven Subjects

FIG.25

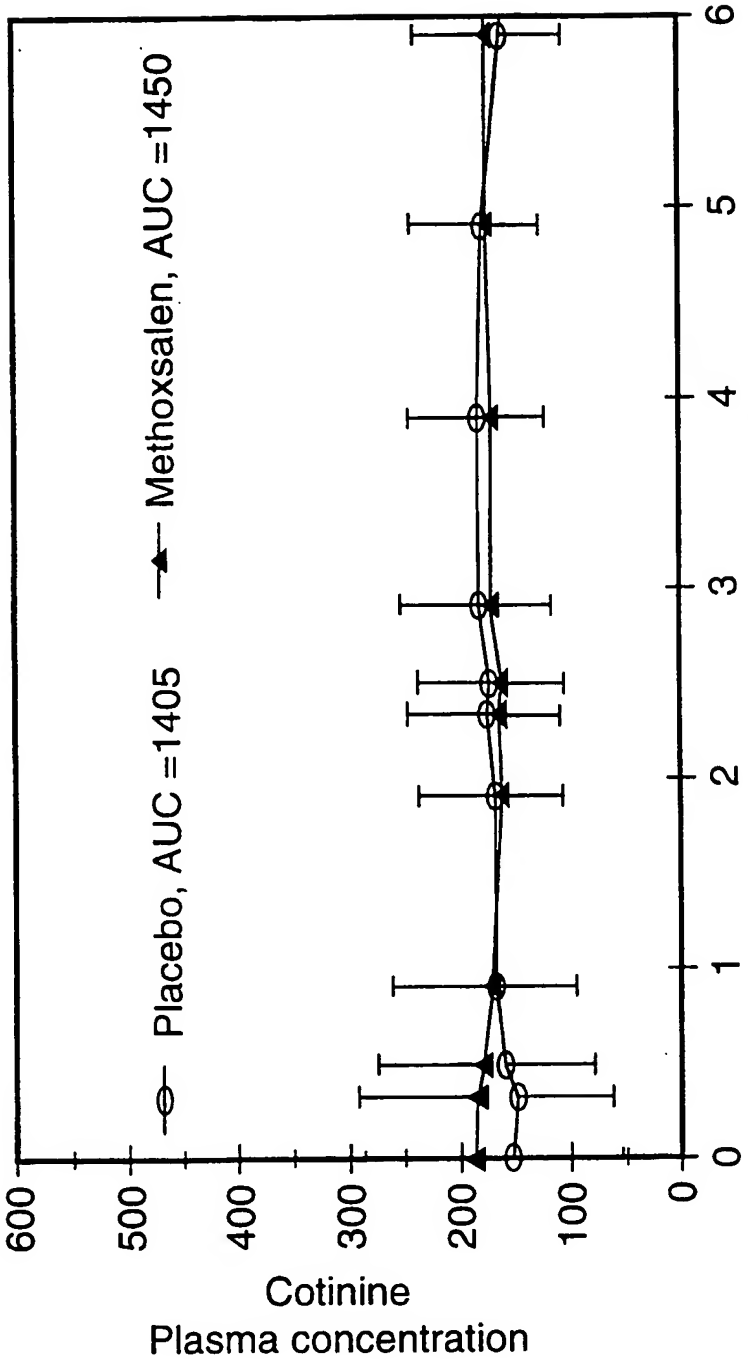

Placebo, AUC =1405
Methoxsalen, AUC =1450
600
500
400
300
200
100
0
0
1
2
3
4
5
6
Cotinine
Plasma concentration
Elapsed time, minutes
Seven subjects

FIG.26

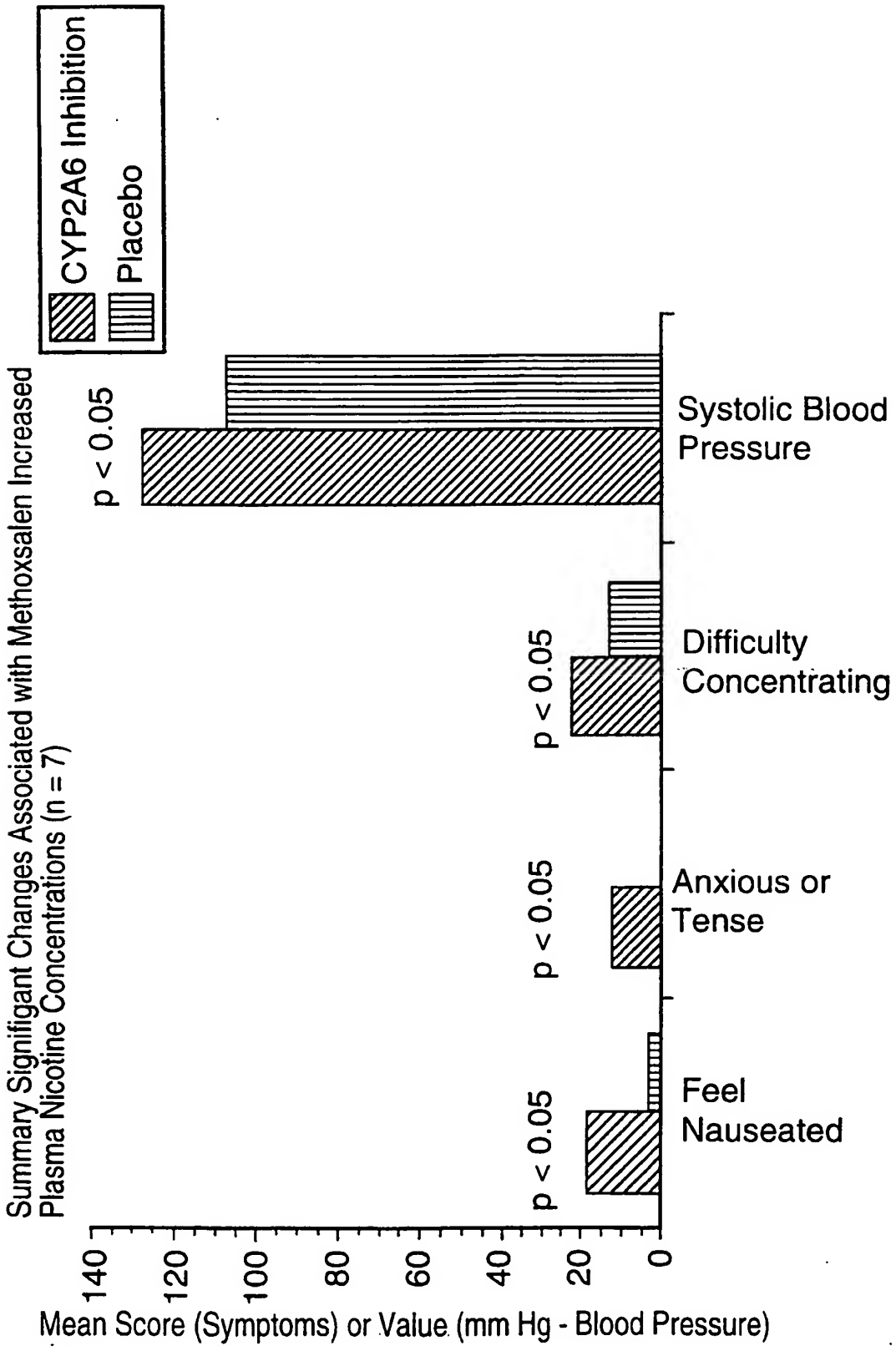

CYP2A6 Inhibition
Placebo
Summary Signifigant Changes Associated with Methoxsalen Increased Plasma Nicotine Concentrations (n = 7)
p < 0.05
Systolic Blood Pressure
p < 0.05
Difficulty Concentrating
p < 0.05
Anxious or Tense
p < 0.05
Feel Nauseated
140
120
100
80
60
40
20
0
Mean Score (Symptoms) or Value (mm Hg - Blood Pressure)

FIG.27

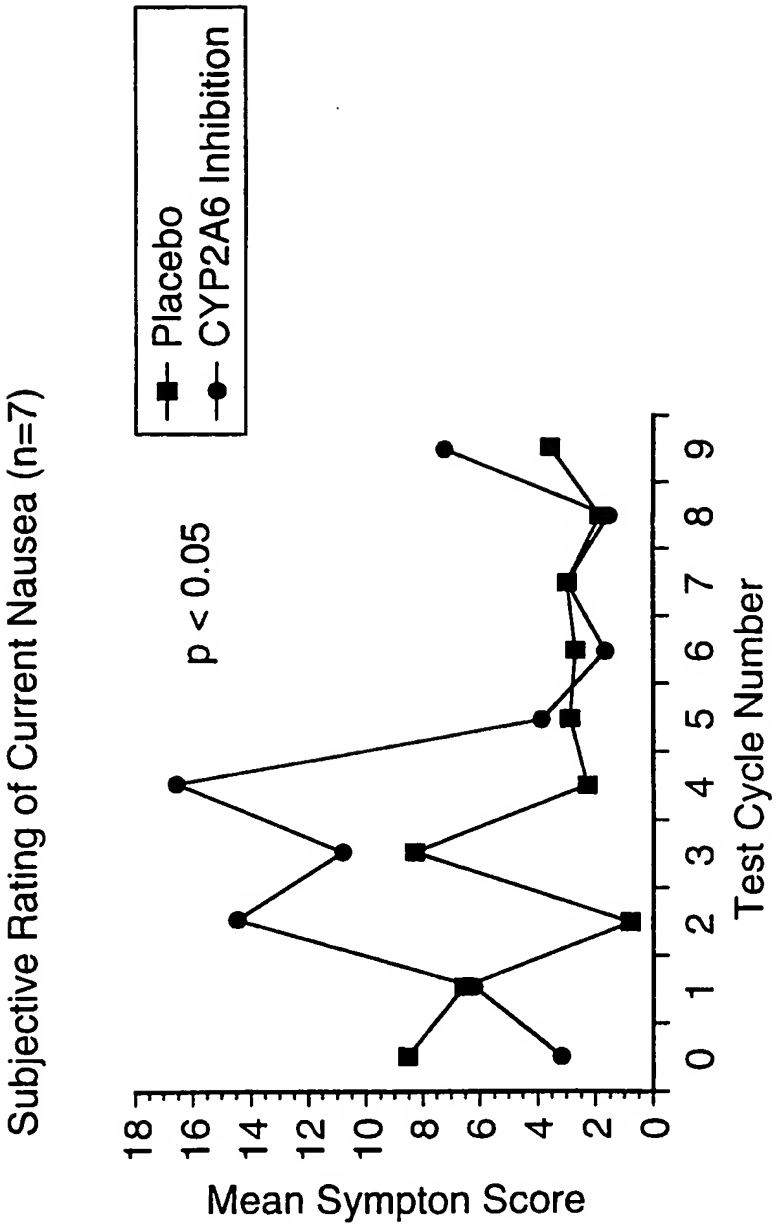

Subjective Rating of Current Nausea (n=7)
Placebo
CYP2A6 Inhibition
p < 0.05
Mean Sympton Score
0
2
4
6
8
10
12
14
16
18
0 1 2 3 4 5 6 7 8 9
Test Cycle Number

FIG.28A

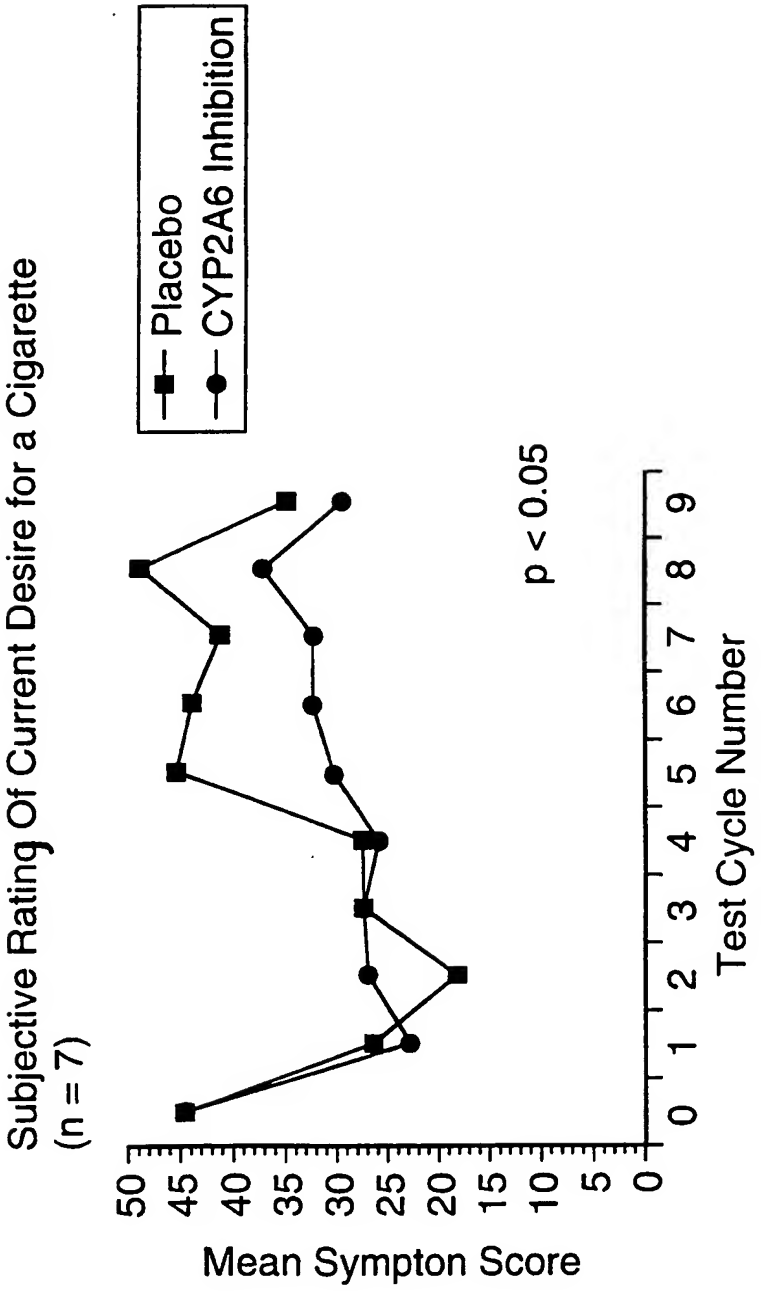

Subjective Rating Of Current Desire for a Cigarette
(n = 7)
Placebo
CYP2A6 Inhibition
p < 0.05
Mean Sympton Score
Test Cycle Number
0
5
10
15
20
25
30
35
40
45
50
0
1
2
3
4
5
6
7
8
9

FIG.28B

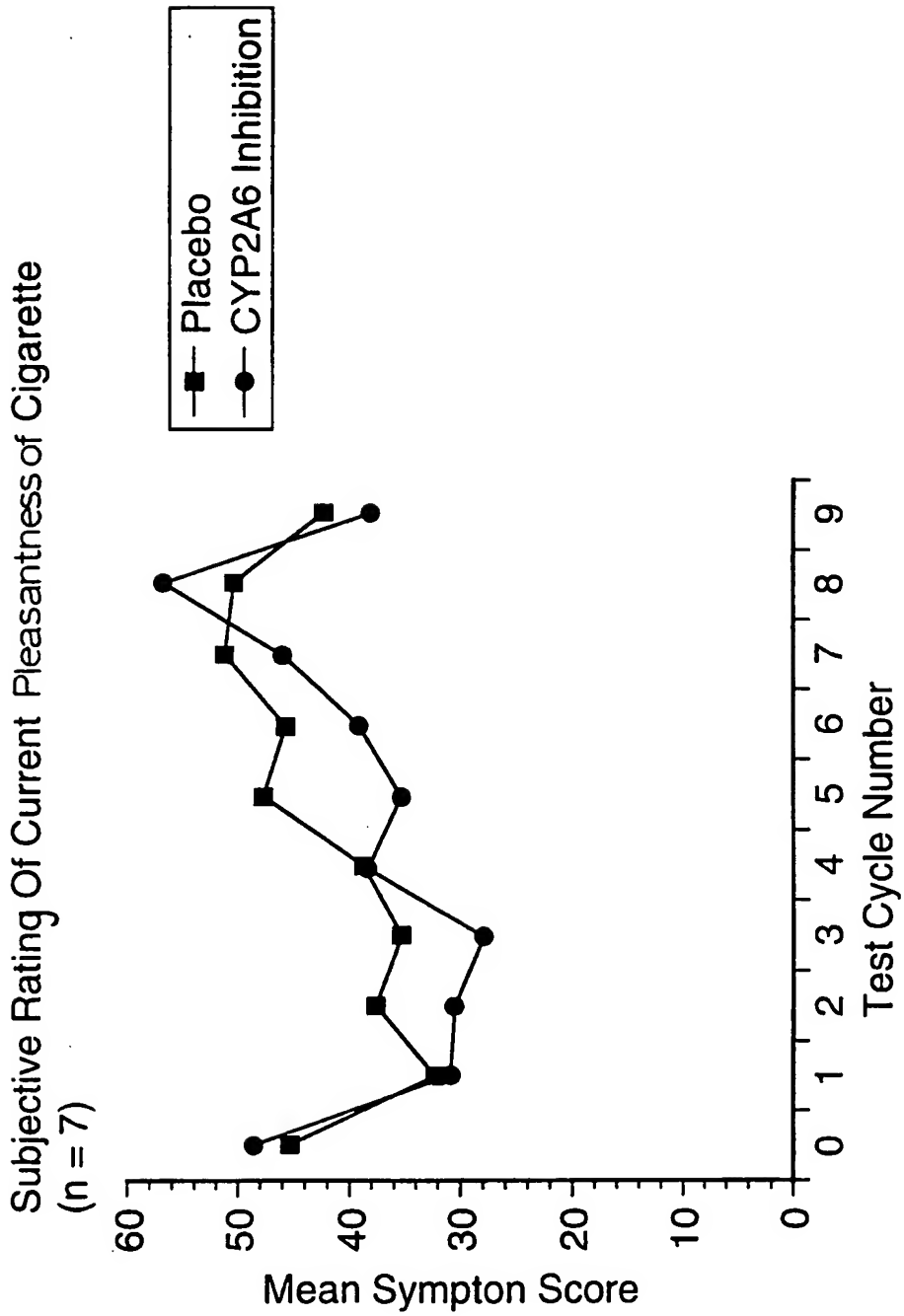

Subjective Rating Of Current Pleasantness of Cigarette
(n = 7)
Placebo
CYP2A6 Inhibition
Mean Sympton Score
0
10
20
30
40
50
60
0 1 2 3 4 5 6 7 8 9
Test Cycle Number

FIG.28C

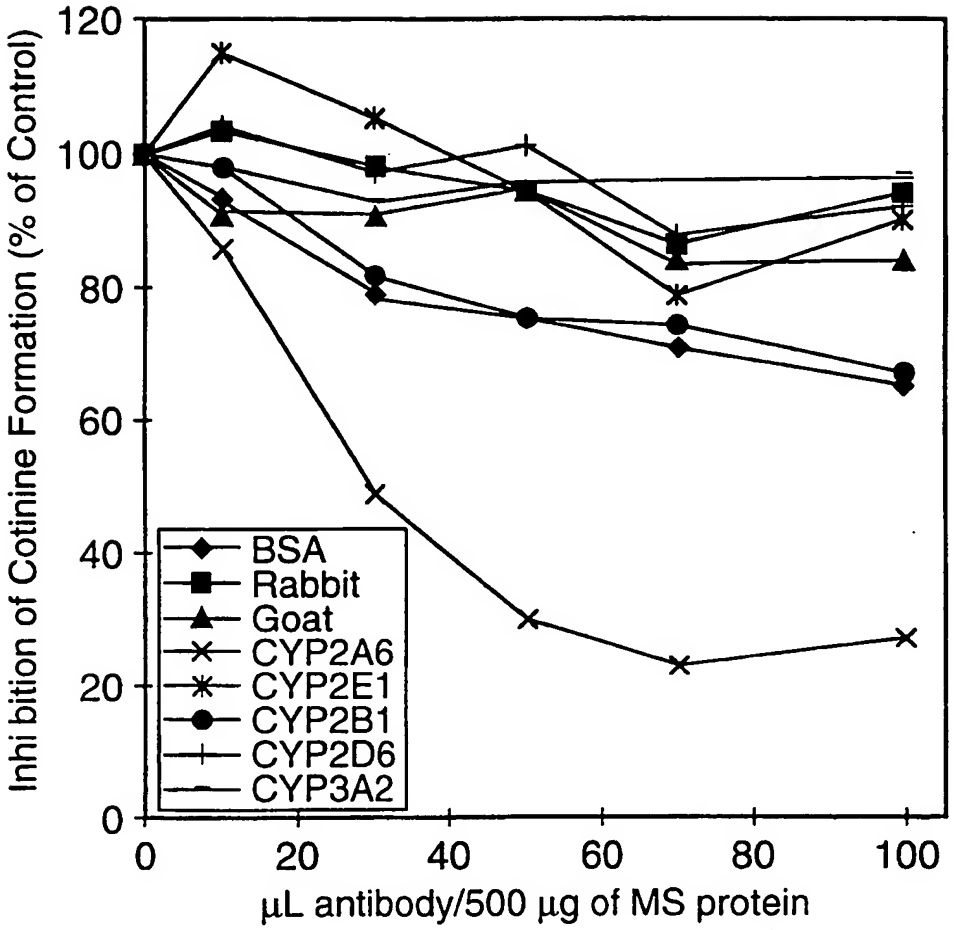

Inhi bition of Cotinine Formation (% of Control)
120
100
80
60
40
20
0
0
20
40
60
80
100
µL antibody/500 µg of MS protein
BSA
Rabbit
Goat
CYP2A6
CYP2E1
CYP2B1
CYP2D6
CYP3A2

FIG.29

Inhibition of Nicotine to Cotinine Metabolism by various Compounds

Inhibitor	Ki	% Inhibition at 10 uM	% Inhibition at 100 uM	% Inhibition at 150 uM
coumarin	2 uM (n+4)	65 (n=1)	90 (n=1)	85 +/- 11 (SD, n=31)
7-methoxycoumarin	2.5 uM (n=1)	40 (n=1)	60 (n=3)	———
7-methylcoumarin	15 uM*	20 (n=1)	70 (n=3)	———
7-ethoxycoumarin	>100 uM*	10 (n=1)	20 (n=3)	———
7-hydroxycoumarin	200 uM	———	25 (n+1	———
diethyldithiocarbamic acid	14.5 uM (n=1)	———	———	———
pilocarpine	0.1 uM	———	———	———
naringenin	4.3 uM (n=1)	30 (n=1)	70 (n=3)	———
methoxsalen	0.02 uM (n=1)	———	———	———
naringin	.100 uM*	———	10 (n=1)	———
bupropion	———	20 (n=1)	30 (n=1)	———
orphenadrine	———	———	———	20 +/- 16 (SD, n=30)
troleandomycin	———	———	———	3 +/- 11 (SD, n=30)

all nicotine concentrations were at the Km value for cotinine formation in their respective livers
** estimated from screening studies with 10 and 100 uM inhibitor concentrations*

FIG.30A

Ki Values for the Inhibition of the CYP2A6 Substrate Coumarin to 7-Hydroxycoumarin Metabolism by various compound

Inhibitor	Human liver	Monkey liver
methoxsalen	0.29 uM	1.69 uM
nicotine	100.1 uM	24.1 uM
pilocarpine	0.9 uM	0.9 uM

FIG.30B

Effect of Various Compounds on Cotinine Formation

	% control cotinine formation	
Inhibitor	10 uM	100 uM
coumarin	35	10
naringenin	70	30
7-methylcoumarin	80	30
7-methoxycoumarin	60	40
bupropion	80	70
7-ethoxycoumarin	90	80

FIG.30C

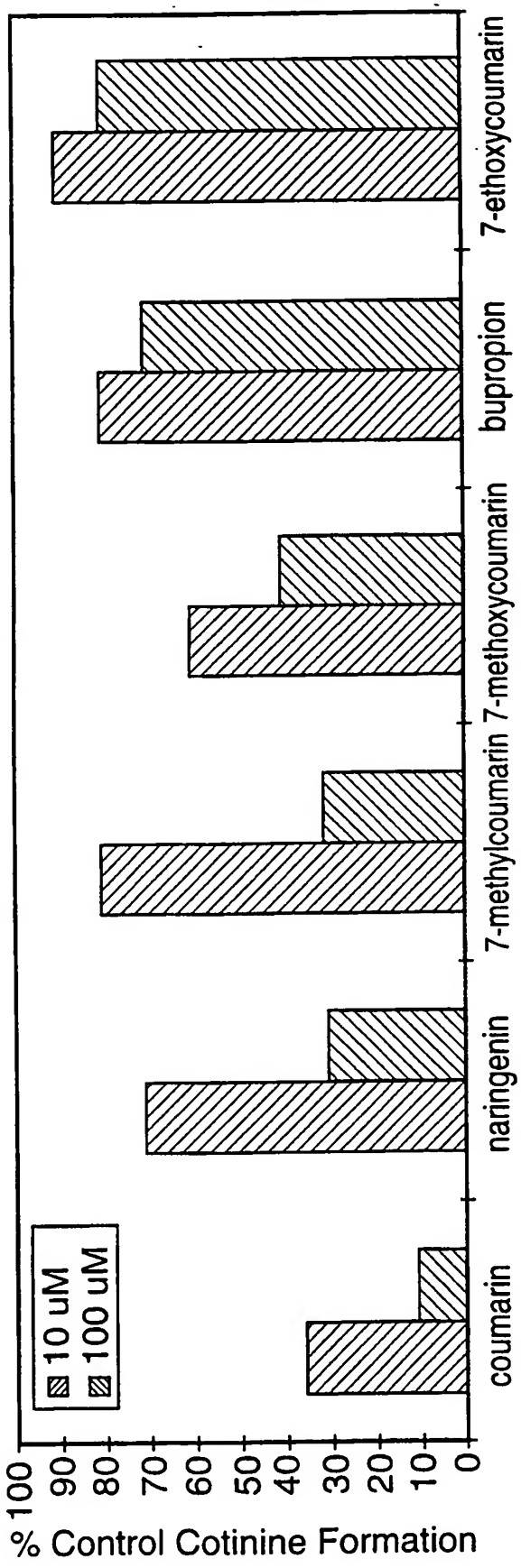

% Control Cotinine Formation
100
90
80
70
60
50
40
30
20
10
0
10 uM
100 uM
coumarin
naringenin
7-methylcoumarin
7-methoxycoumarin
bupropion
7-ethoxycoumarin

FIG.30D

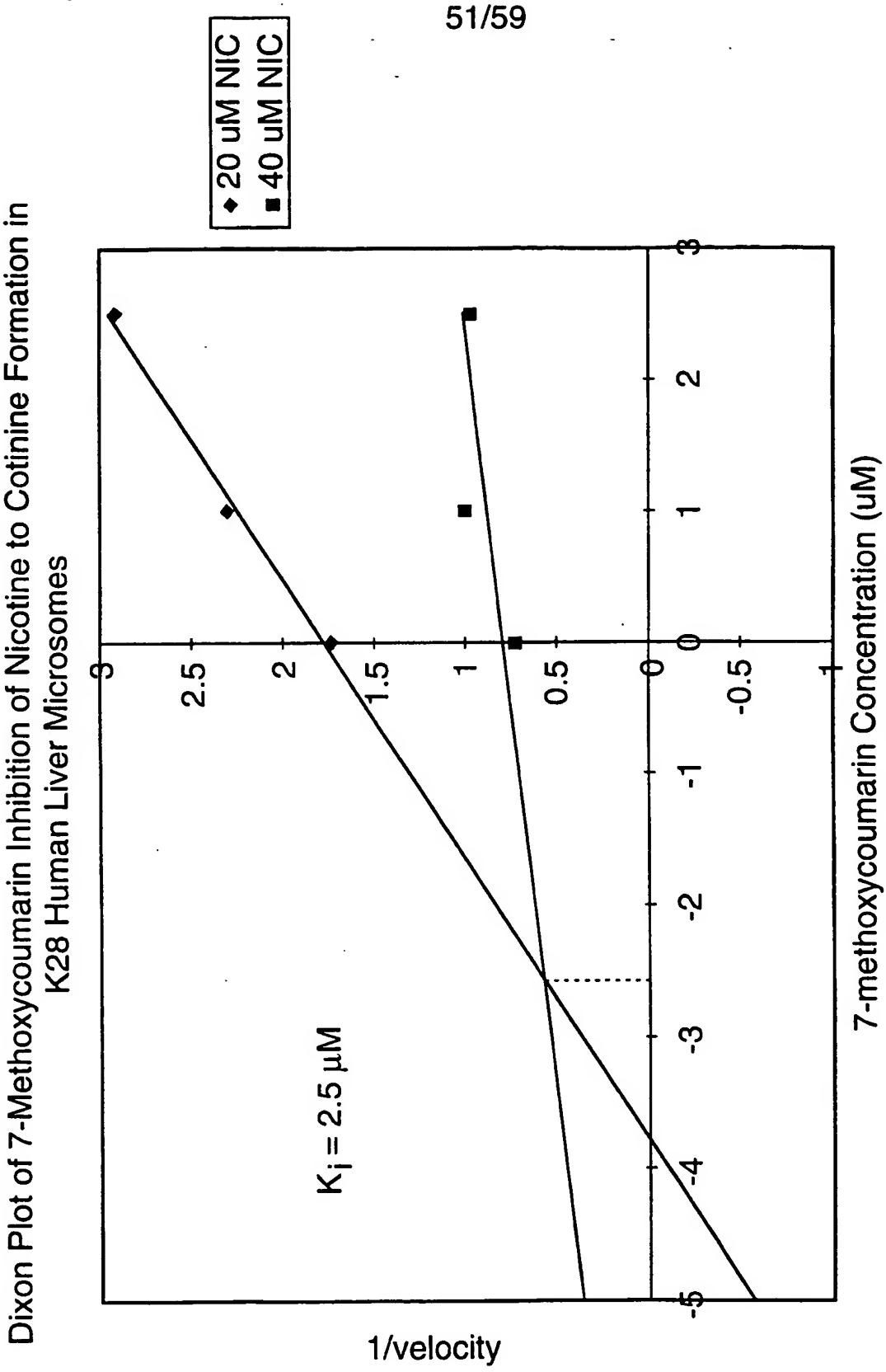

Dixon Plot of 7-Methoxycoumarin Inhibition of Nicotine to Cotinine Formation in K28 Human Liver Microsomes
20 uM NIC
40 uM NIC
K_i = 2.5 µM
1/velocity
7-methoxycoumarin Concentration (uM)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
3
2
1
0
-1
-2
-3
-4
-5

FIG.31

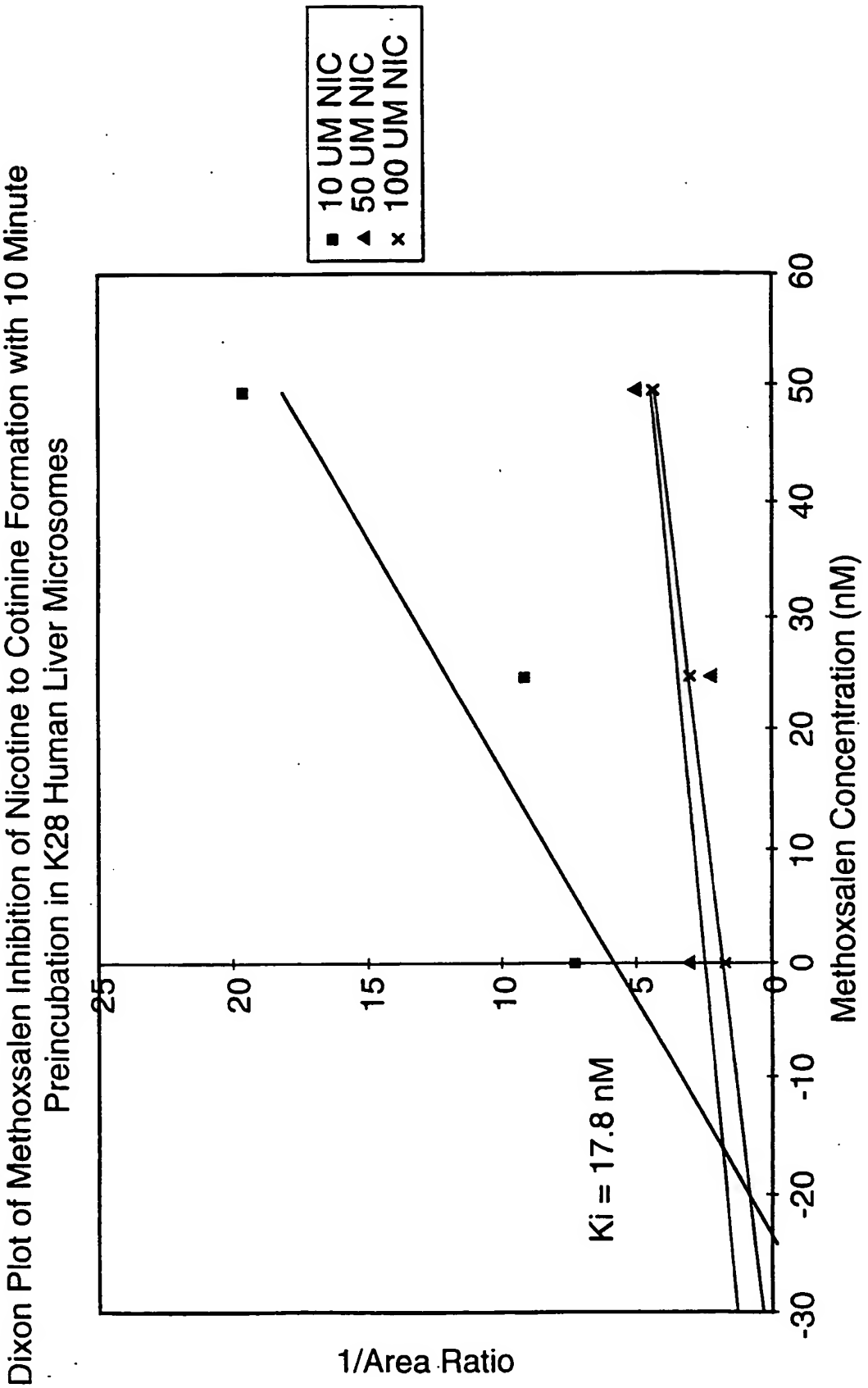

Dixon Plot of Methoxsalen Inhibition of Nicotine to Cotinine Formation with 10 Minute Preincubation in K28 Human Liver Microsomes
10 UM NIC
50 UM NIC
100 UM NIC
Ki = 17.8 nM
1/Area Ratio
Methoxsalen Concentration (nM)
25
20
15
10
5
0
60
50
40
30
20
10
0
-10
-20
-30

FIG.32

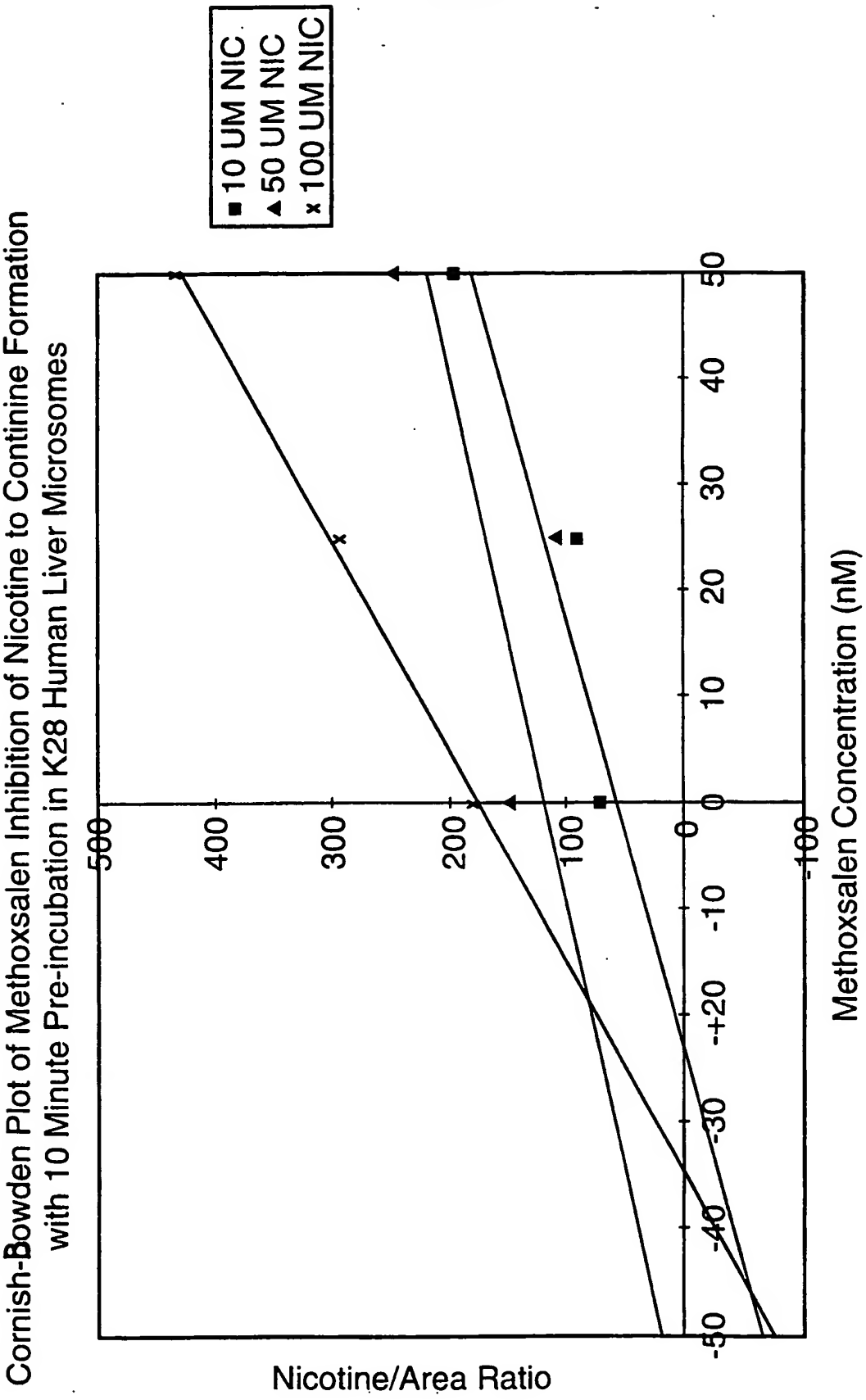

Cornish-Bowden Plot of Methoxsalen Inhibition of Nicotine to Continine Formation
with 10 Minute Pre-incubation in K28 Human Liver Microsomes
■ 10 UM NIC
▲ 50 UM NIC
x 100 UM NIC
Nicotine/Area Ratio
500
400
300
200
100
0
-100
Methoxsalen Concentration (nM)
50
40
30
20
10
0
-10
-+20
-30
-40
-50

FIG.33

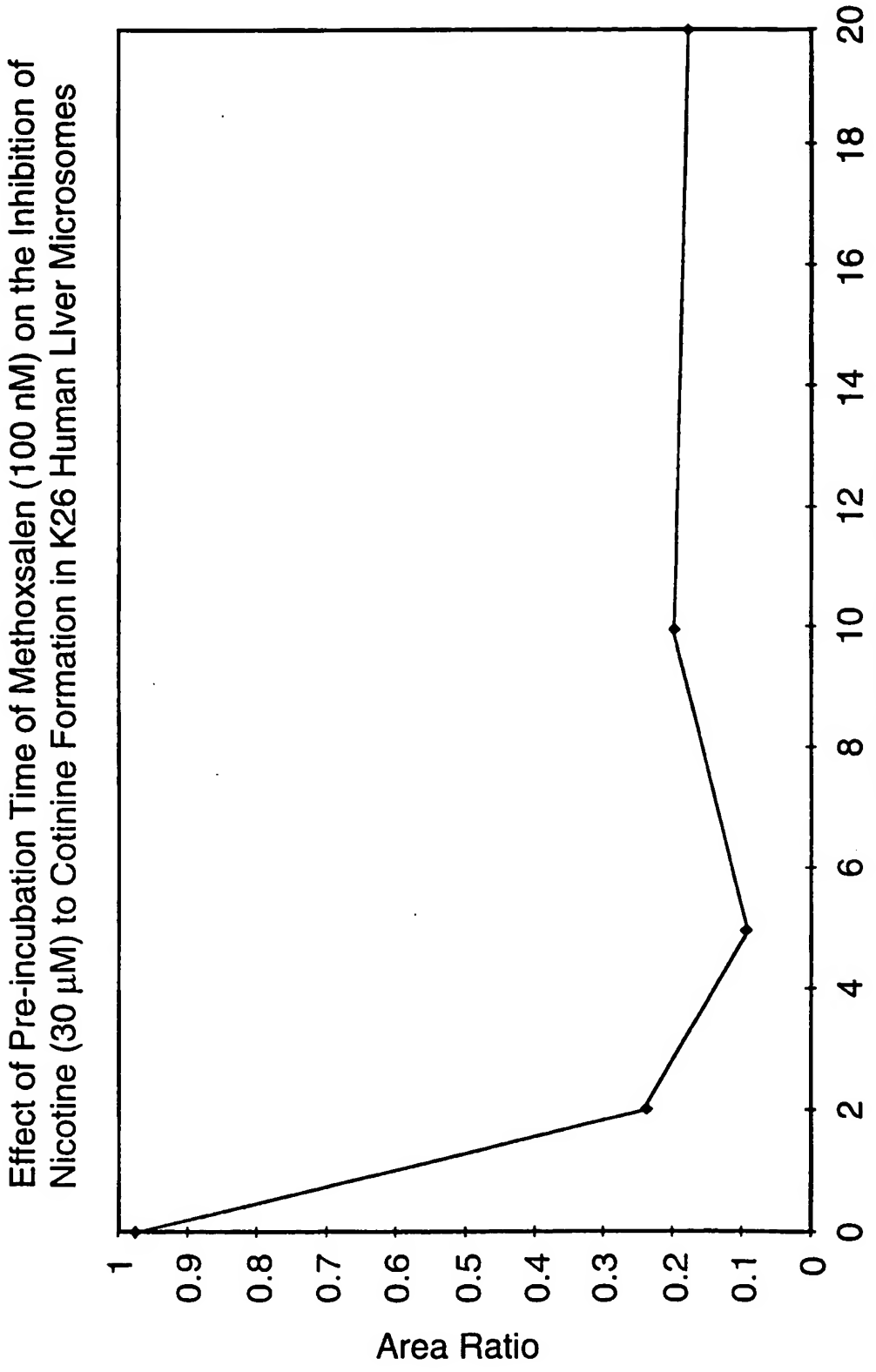

Effect of Pre-incubation Time of Methoxsalen (100 nM) on the Inhibition of Nicotine (30 µM) to Cotinine Formation in K26 Human Liver Microsomes
Area Ratio
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
2
4
6
8
10
12
14
16
18
20
Pre-incubation Time(min)

FIG.34

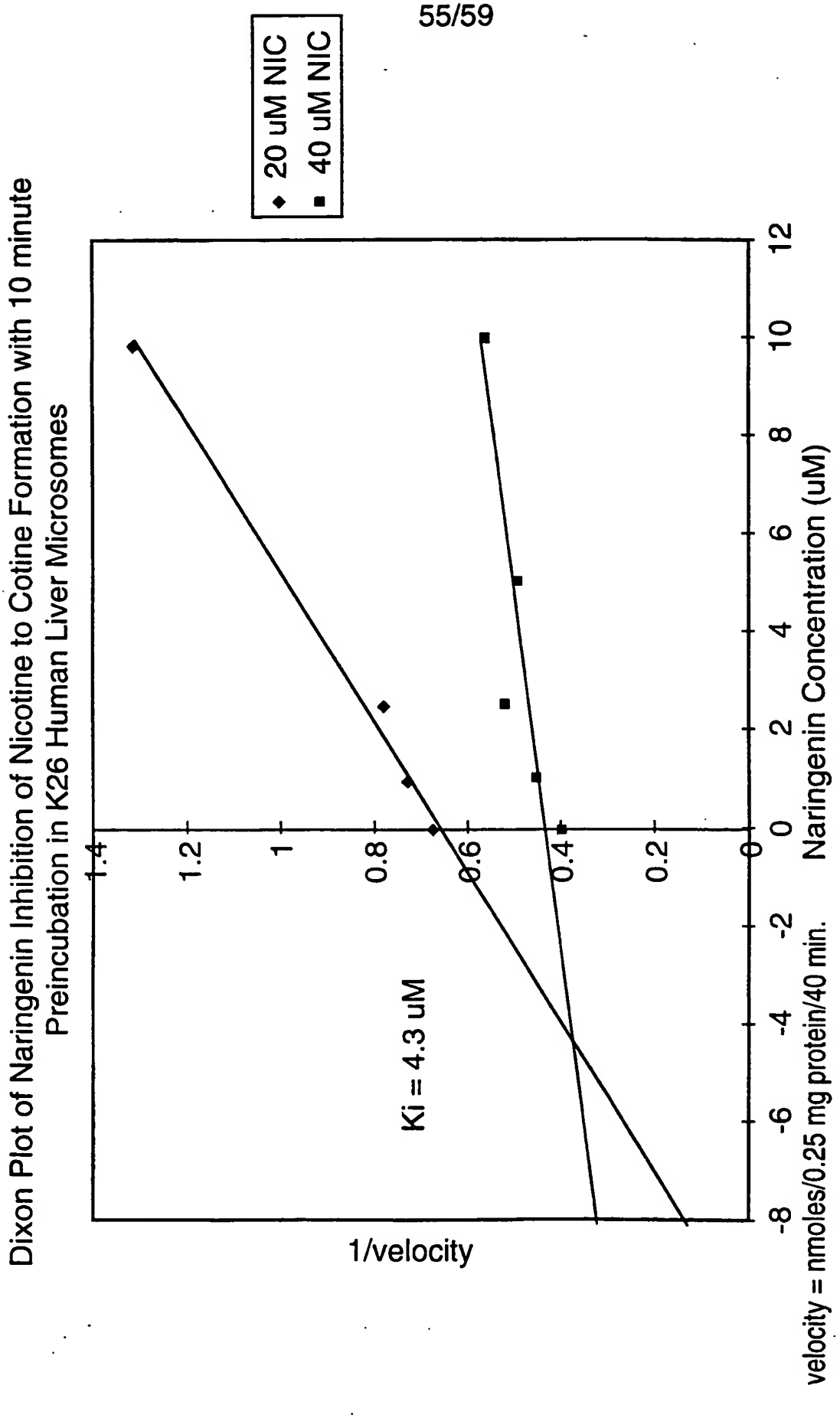

Dixon Plot of Naringenin Inhibition of Nicotine to Cotine Formation with 10 minute Preincubation in K26 Human Liver Microsomes
20 uM NIC
40 uM NIC
Ki = 4.3 uM
1/velocity
velocity = nmoles/0.25 mg protein/40 min.
Naringenin Concentration (uM)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-8
-6
-4
-2
0
2
4
6
8
10
12

FIG.35

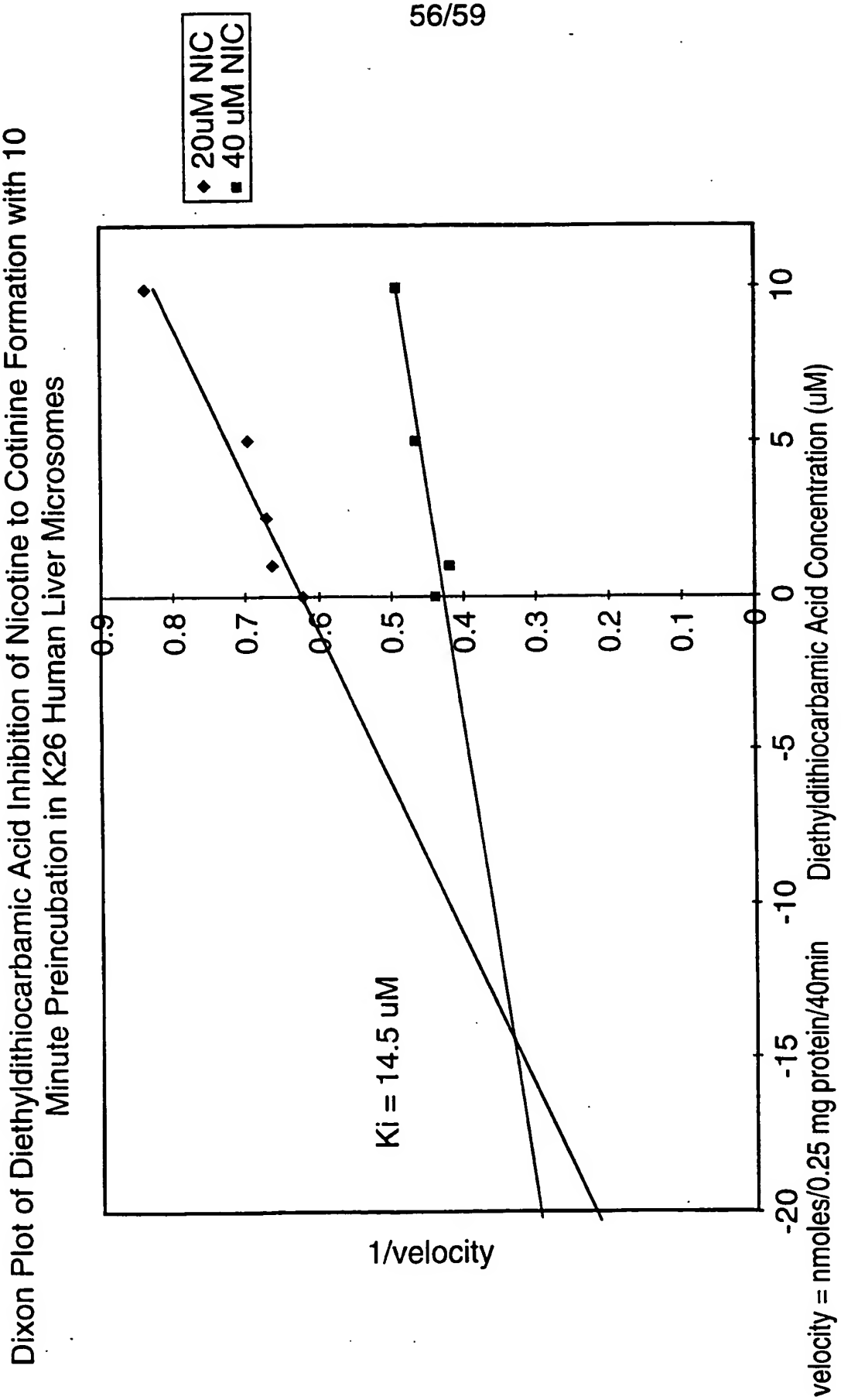

Dixon Plot of Diethyldithiocarbamic Acid Inhibition of Nicotine to Cotinine Formation with 10 Minute Preincubation in K26 Human Liver Microsomes
20uM NIC
40 uM NIC
Ki = 14.5 uM
1/velocity
velocity = nmoles/0.25 mg protein/40min
Diethyldithiocarbamic Acid Concentration (uM)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-20
-15
-10
-5
0
5
10

FIG.36

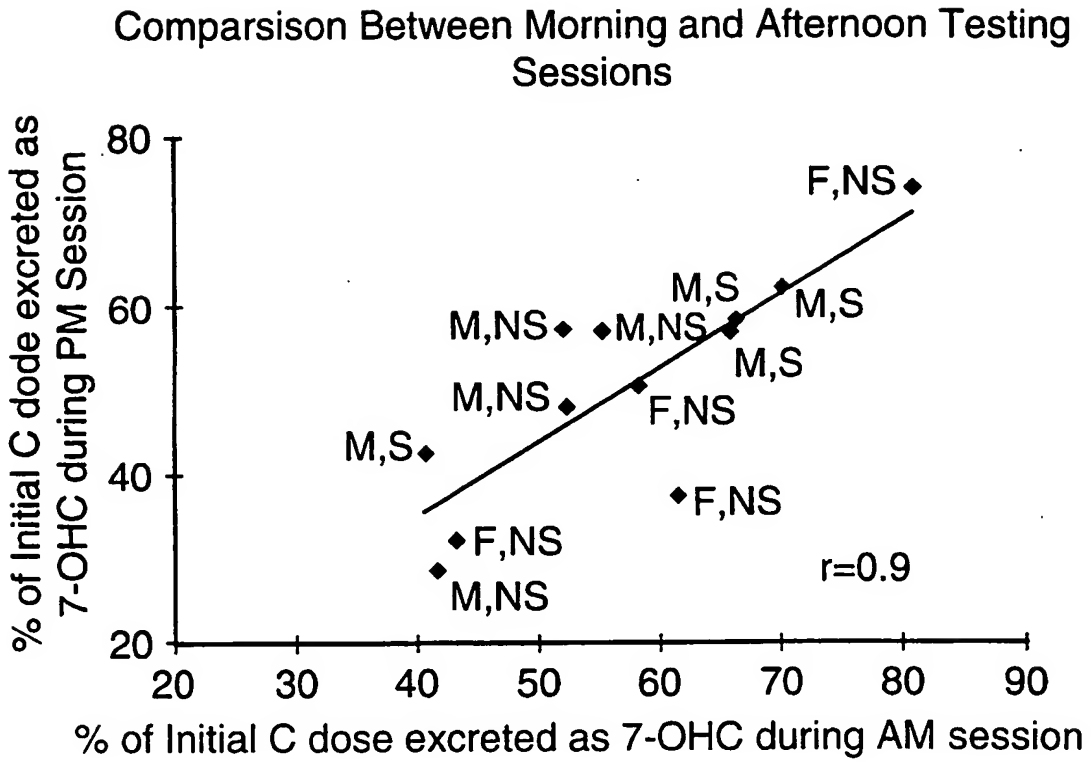

Comparsison Between Morning and Afternoon Testing Sessions
% of Initial C dode excreted as 7-OHC during PM Session
80
60
40
20
20
30
40
50
60
70
80
90
% of Initial C dose excreted as 7-OHC during AM session
F,NS
M,S
M,S
M,NS
M,NS
M,S
M,NS
F,NS
M,S
F,NS
F,NS
M,NS
r=0.9

FIG.37

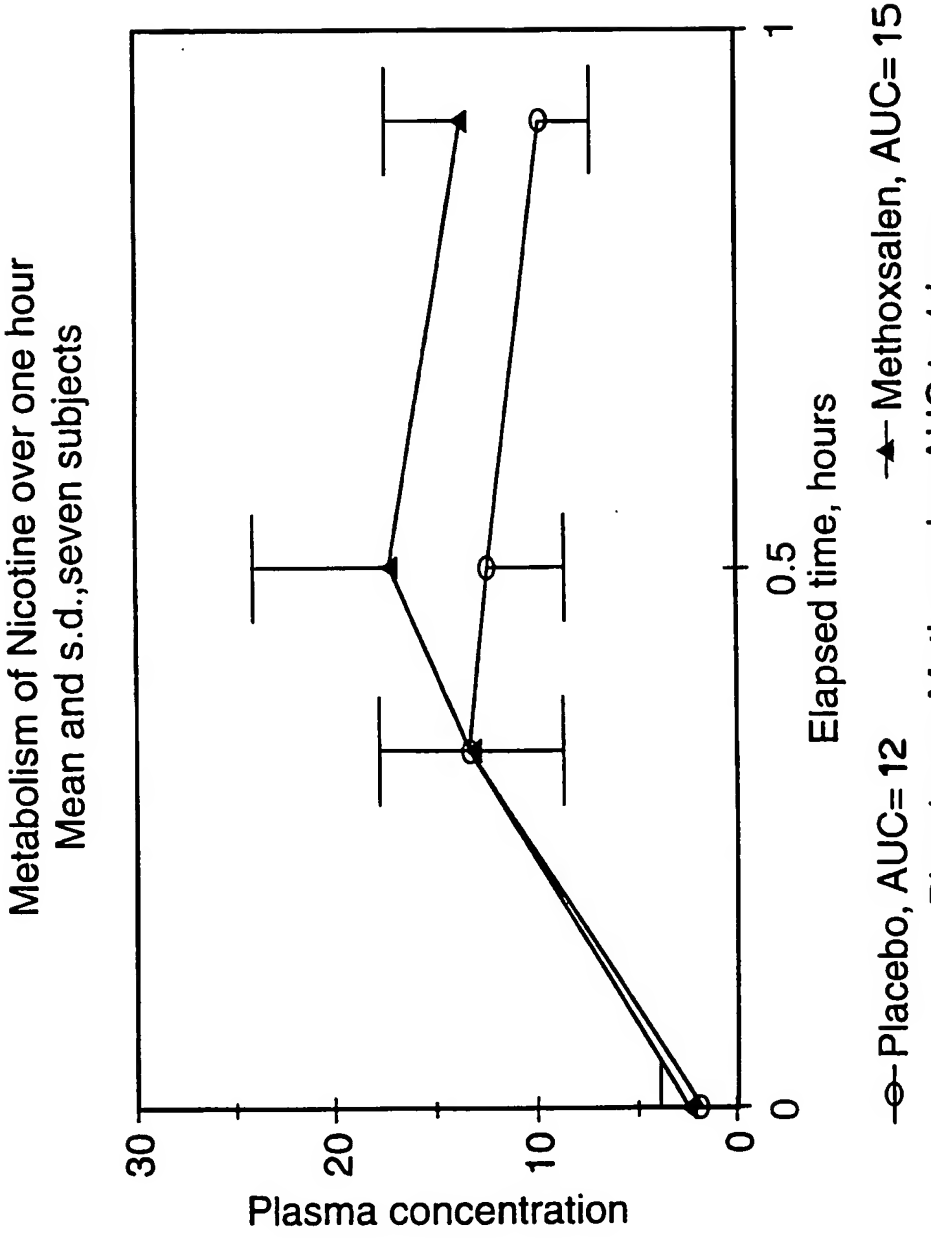

Metabolism of Nicotine over one hour
Mean and s.d.,seven subjects
Plasma concentration
30
20
10
0
0
0.5
1
Elapsed time, hours
Placebo, AUC= 12
Methoxsalen, AUC= 15
Placebo vs Methoxsalen AUC to 1 hour:
F(1,6)=8.07,p=0.0295

FIG.38

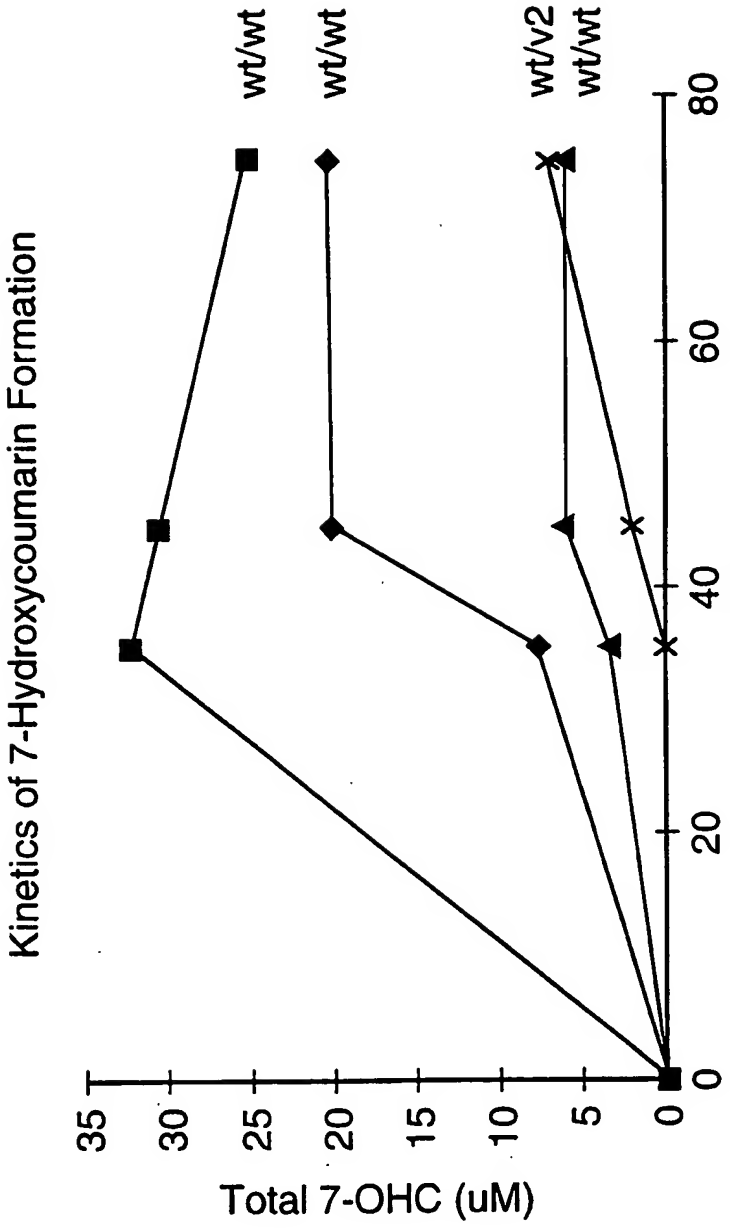

Kinetics of 7-Hydroxycoumarin Formation
wt/wt
wt/wt
wt/v2
wt/wt
Total 7-OHC (uM)
35
30
25
20
15
10
5
0
0
20
40
60
80
Time (mins)

FIG.39